

Environmental and genetic predictors
of reproductive success and sex ratios in vertebrates

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Ph.D. thesis

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Environmental and genetic predictors of reproductive success and sex ratios in vertebrates
(A szaporodási sikert és ivarárányt befolyásoló környezeti és genetikai tényezők)

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Abstracts

Abstract in English

Environmental and genetic predictors of reproductive success and sex ratios in vertebrates

Recent climate change and the alteration of natural habitats are among the most important forms of human-induced environmental change. Adaptation to the increasingly frequent extreme weather events is challenging, and potentially threatening for wild animal populations. The growing number and area of cities alter ecological and climatic characteristics for wild animals, which can also threaten the persistence of animal populations. Moreover, the success of a population can be influenced by the sex ratio of the population, both in juvenile and adult age groups.

During my doctoral studies, I investigated the effects of extreme weather events and habitat urbanisation on the reproductive success and sex ratios of bird populations. I also investigated the causes of interspecific variation in sex ratio, a fundamental element of the social environment of individuals, in vertebrate populations. Using correlative field studies I showed that extreme weather events can have diverse effects on reproductive output of birds depending on the investigated component of reproductive success and habitat type. I found that average and extreme hot temperatures had opposite effects on body mass and tarsus length of house sparrow (*Passer domesticus*) offspring. Average body size of nestlings increased with higher average temperature, but decreased with increasing number of hot days. However, hatching success was influenced positively by the number of hot days. In great tit (*Parus major*) populations, number of hot days was less harmful for the nestlings in warmer urban sites than in natural forest sites, as the body mass of broods decreased more intensively with increasing number of hot days in forests than in urban habitats. The frequency of extra-pair offspring occurrence was significantly higher in urban than in forest great tit populations. This means that urban great tit females cuckold more often than their forest conspecifics.

Brood sex ratios were balanced in both species. Offspring sex ratio did not differ between habitat types in great tits and did not correlate with weather conditions in house sparrows. In a phylogenetic comparative study of vertebrate species, I found a significant relationship between the direction of adult sex ratio bias and the type of genetic sex-determination system. In male-heterogametic species (with XY system) adult sex ratio was more male-biased than in female-heterogametic species (ZW system). My results contribute to understanding the effects of two global environmental changes, and highlight the importance of studying sex ratios of wild animal populations.

Összefoglaló magyarul

A szaporodási sikert és ivararányt befolyásoló környezeti és genetikai tényezők

Az emberi tevékenység által okozott két legjelentősebb környezeti változás a globális klímaváltozás és a természetes élőhelyek átalakítása. A klímaváltozással együtt járó egyre gyakoribb szélsőséges időjárási eseményekhez való alkalmazkodás kihívás elé állítja a vadon élő állatpopulációkat, veszélyeztetve akár azok fennmaradását is. A városok száma és kiterjedése növekszik, az itt élő vadállatok számára ez a környezet új ökológiai és klimatikus viszonyokkal jellemezhető, az ehhez való alkalmazkodás sikerességétől függhet a városi populációk fennmaradása. A populációk sikeressége függhet attól is, hogy mennyire kiegyenlített vagy variábilis a populációban az ivararány, mind fiatal, mind felnőtt korban.

Doktori tanulmányaim során a szélsőséges időjárási események és az élőhely-urbanizáció állatpopulációk szaporodási sikerességére és demográfiai tulajdonságaira gyakorolt hatásait vizsgáltam, valamint a szociális környezet egyik alapvető elemének, az ivararány fajok közötti változatosságának okait kutattam. Korrelatív terepi vizsgálatokkal kimutattam, hogy a szélsőséges időjárási események változatosan hathatnak a madarak szaporodási sikerességére a vizsgált szaporodási siker komponenstől és az élőhelytől függően. Azt találtam, hogy az átlagos és extrém hőmérsékletek hatása ellentétes a házi veréb (*Passer domesticus*) fiókák testméretére: az átlaghőmérséklet emelkedésével a verébfiókák testtömege és csüd hossza nagyobb volt, míg a hőségnapok számának növekedése kedvezőtlenül hatott. A kelési siker viszont növekedett a hőségnapok számával. Széncinege (*Parus major*) populációkban a melegebb városi területeken a fiókák számára kevésbé volt káros az extrém hőség az erdei fajtársaikhoz képest: az erdei fészekaljokban a fiókatömeg nagyobb mértékben csökkent a hőségnapok számával, mint a városi fészekaljokban. A városi széncinegékénél ezen kívül magasabb extra-pár utód előfordulási gyakoriságot találtam, azaz a városi madarak gyakrabban lépnek félre, mint erdei társaik. Az utódok ivararánya mindkét vizsgált madárfajnál kiegyenlített volt, nem különbözött a városi és erdei széncinege fészekaljok között, és házi verebeknél nem függött össze az időjárási körülményekkel.

Gerinces fajok filogenetikai összehasonlító vizsgálatával szignifikáns összefüggést mutattam ki a felnőttkori ivararány eltolódásának iránya és a genetikai ivardeterminációs módok között. Azoknál a fajoknál, melyeknél a hím a heterogametikus ivar (XY kromoszóma rendszer), a felnőttkori ivararány nagyobb mértékben a hímek felé eltolódott, mint azoknál a fajoknál, ahol a nőstény a heterogametikus (ZW kromoszóma rendszer). Eredményeim hozzájárulnak két világméretű környezeti változás hatásainak jobb megértéséhez, valamint hangsúlyozzák a populációs ivararányok vizsgálatának fontosságát.

Zusammenfassung auf Deutsch

Umwelt- und genetische Prädiktoren für den Fortpflanzungserfolg und das Geschlechterverhältnis bei Wirbeltieren

Der Klimawandel und die Urbanisation gehören zu den wichtigsten Formen des Umweltwandels vom Menschen. Die Anpassung an die immer häufiger auftretenden extremen Wetterereignisse ist potenziell bedrohlich für Wildtierpopulationen. Die wachsende Anzahl und Fläche von Städten verändert die ökologischen und klimatischen Eigenschaften von Wildtieren. Der Erfolg einer Spezies kann durch das Geschlechterverhältnis beeinflusst werden.

Während meiner Studien habe ich die Auswirkungen von extremen Wetterereignissen und der Urbanisation von Lebensräumen auf den Fortpflanzungserfolg und das Geschlechterverhältnis von Vogelpopulationen untersucht. Ich untersuchte auch die Ursachen für interspezifische Variationen des Geschlechterverhältnisses. Ich demonstrierte mit korrelativen Feldstudien, dass extreme Wetterereignisse an mehrere Komponente des Fortpflanzungserfolgs einwirken können. Die Effekte können Abhängig von der Lebensraumtyps in Vögeln werden. Ich fand heraus, dass die Körpermasse und die Tarsuslänge von juvenil Haussperlingen (*Passer domesticus*) bei durchschnittliche und extrem heiße Temperaturen gegensätzlich beeinflusst werden. Die durchschnittliche Körpergröße der Jungen nahm mit steigender Durchschnittstemperatur zu, nahm jedoch mit zunehmender Anzahl heißer Tage ab. Der Schlupferfolg wurde durch die Anzahl der heißen Tage positiv beeinflusst. In Kohlmeisenpopulationen (*Parus major*) war die Anzahl der heißen Tage für die Junge in wärmeren städtischen Gebieten weniger schädlich als in natürlichen Waldgebieten, da die Körpermasse der Brut mit zunehmender Anzahl heißer Tage in Wäldern stärker abnahm als in städtischen Lebensräumen. In Kohlmeisen war die Häufigkeit der Fehlritte in Städte signifikant höher als im Wald. Die Geschlechterverhältnisse bei Junge waren bei beiden Vogelspezies ausgeglichen. Das Geschlechterverhältnis bei Jungen unterschied sich nicht zwischen den Lebensraumtypen bei Kohlmeisen und korrelierte nicht mit den Wetterbedingungen bei Haussperlingen.

In einer phylogenetischen Studie mit Wirbeltiere fand ich einen signifikanten Zusammenhang zwischen der Richtung der Verzerrung des Geschlechterverhältnisses bei Erwachsenen und die genetischen Geschlechtschromosomen. Bei männlich-heterogametischen Spezies (mit XY-system) war das Geschlechterverhältnis bei Erwachsenen stärker männlich voreingenommen als bei weiblich-heterogametischen Spezies (ZW-system). Meine Ergebnisse tragen zum besser Verständnis der Auswirkungen zweier globaler Umweltveränderungen bei, und unterstreichen die Bedeutung der Untersuchung des Geschlechterverhältnisses von Wildtierpopulationen.

1. General introduction

1.1. Effects of human-induced environmental changes on reproduction

Humans and human activity is increasingly present almost everywhere across the globe (United Nations 2014) even in protected natural areas (Blumstein *et al.* 2017), and human activity changes also the environment of every other organisms, biodiversity loss is increasing rapidly as a consequence (Maxwell *et al.* 2016). Humans induce environmental changes in several ways, including constructions of buildings and roads, deforestation, fishing, agriculture and making large garbage despositories. It seems species can persist only when they adapt to these human-induced changes.

One of the greatest human-induced changes of the whole ecosystem is the recent climate change (i.e. greenhouse gas driven). Exploring the effects of climate change is one of the hottest topics of recent research in ecology. Earth's global surface temperature is increasing, 2018 was the fourth warmest year since 1880 (by 0.83 °C warmer than the 1951 to 1980 mean), according to independent analyses by National Oceanic and Atmospheric Administration (NOAA) and NASA (<https://climate.nasa.gov/>). How organisms react to climate change mainly depends on their adaptive capacity including phenological and/or physiological adaptation, migration and colonisation of new habitats, and additionally, timing and magnitude of these adaptations to the altered environment are important constraints for success of species' responses (European Environment Agency 2012). The ecological consequences of the increase in average temperature include 1) seasonal and phenological shifts as spring and summer phenologies start earlier and growing/breeding seasons last longer, 2) distributional shifts that are usually poleward (latitudinal) and upward (altitudinal) range shifts, including pest and disease shifts to higher latitudes, 3) changing interactions across trophic levels, as interacting species responding differently to climate warming become more and more asynchronous, and 4) species extinctions (reviewed in Parmesan 2006). We are in Earth's sixth mass extinction period now, about 8 % of species are predicted to become extinct in the near future due to climate change according to a meta-analysis (Urban 2015), and 32% (8,851/27,600) of known vertebrate species have already decreasing population size and range (Ceballos, Ehrlich & Dirzo 2017), which will probably have negative cascading consequences on ecosystem functioning.

The effects and ecological consequences of extreme weather events (e.g. extreme hot days, cold spells, sudden heavy rainfalls, long droughts) have been much less investigated

so far than the consequences of changes in mean temperature (Jentsch & Beierkuhnlein 2008; van de Pol *et al.* 2017), but the frequency of such extreme events increased in recent decades (Buckley & Huey 2016) and will likely increase in the future according to climate models (Field *et al.* 2012). Extreme weather events are rare, but their impact on human society and biological systems is potentially high, e.g. health damage and mortality related to heat and/or cold are increasingly affecting human populations worldwide (Robine *et al.* 2008; Bi *et al.* 2011; Chen *et al.* 2016; Heaviside *et al.* 2016) but see (Chung *et al.* 2017), as well as domestic livestock (Morignat *et al.* 2014; Cox *et al.* 2016). Studies about how wild animal species are affected by extreme weather were scarce but recently started to increase, both those that investigate the effects of a specified weather event e.g. the European heat wave in 2003 on bird populations (Jiguet *et al.* 2006) and on benthic species (Garrabou *et al.* 2009), and those that investigate population changes related to extreme weather events using long-term data (Gardner *et al.* 2017; Tanner *et al.* 2017). Effects of extreme weather events on single reproductive attempts can have serious short-term consequences (Jentsch & Beierkuhnlein 2008; Palmer *et al.* 2017), as well as influence on the long-term fitness of individuals and even the viability of populations (Møller, Rubolini & Lehikoinen 2008; Salido *et al.* 2012). Despite the growing number of studies, we know little about the long-term consequences of extreme weather events on populations, but it is suggested that not all species respond similarly to an extreme weather event even when closely related groups are investigated (Palmer *et al.* 2017), so reaching general conclusions about biological responses to climate extremes seems difficult (Buckley & Huey 2016; Solow 2017). Due to the species specific responses to extreme weather events and the low number of earlier research, there is a great need for future studies to investigate the effects of extreme weather in natural populations of several different taxa.

Another significant human-induced environmental change beside climate change is habitat urbanisation. Increasing proportion of natural habitats are transformed into built-up areas; more than half of the world's human population lives in cities by now (United Nations *et al.* 2014). Cities cover only 3% of Earth's land surface, but urbanization is now the third most common cause of species endangerment (Maxwell *et al.* 2016). Urban areas are usually characterized by seriously altered nutrient cycles, highly elevated pollution levels, extreme anthropogenic landscape transformations with high amount of artificial and impermeable surfaces. These features have significant impacts on biodiversity and ecosystems (Pickett *et al.* 2011), altering the properties of both animal

and plant communities as some species thrive in urban habitats while others cannot persist there (Seress & Liker 2015).

The most important human impact on the local climate of cities is known as the urban heat island (UHI) effect, reflecting the temperature difference between an urban area and the rural surroundings (Landsberg 1981), which is largely due to heat storage in buildings and sealed roads. The intensity of the urban heat island effect can exceed in magnitude the warming of the climate (up to 10° C), so the cities are preceding the natural areas with the warming process. For example, urbanization through the urban heat island can increase the effect of heat waves and the intensity of heat stress in urban human populations (Hardy 2010). More densely built-up areas are increasingly affected by heat waves compared to surroundings (Tan *et al.* 2010; Gabriel & Endlicher 2011), and European cities in cooler northern climate are more sensitive to additional heat according to a review (Ward *et al.* 2016). On the other hand, urban heat island effect can reduce the effects of extreme cold periods (Whitehouse *et al.* 2013). *It is therefore possible that the impact of weather variability and extreme meteorological events on animal communities is different in urban areas than in non-urban environments, but according to my knowledge, this hasn't been investigated yet.* Detailed information about how urban and natural populations resemble each other or differ in their response to weather conditions would give useful knowledge for urban ecology, urban planning and conservation as well.

Urban animals often differ from conspecifics living in natural habitats in their behaviour, life history, and demography (Miranda *et al.* 2013; Gil & Brunn 2013; Rodewald & Gehrt 2014; Seress & Liker 2015). In birds, many species successfully colonized urban areas worldwide, and urban individuals have to cope with several anthropogenic environmental alterations such as light, noise and chemical pollution (Seress & Liker 2015), habitat fragmentation (Crooks, Suarez & Bolger 2004), and ecological challenges such as higher population densities (Moller *et al.* 2012) and lower availability of natural food (Seress *et al.* 2018). These differences between urban and natural habitats may alter the costs and benefits of birds' reproductive decisions, thereby affecting their reproductive behaviour in urban environments. In line with this, urban birds typically show altered reproductive biology including advanced laying dates, reduced brood size, higher nest-failure rates and smaller nestlings (Chamberlain *et al.* 2009; Bailly *et al.* 2016; Seress *et al.* 2018) compared to their non-urban conspecifics.

There are many components of reproductive success that are much more rarely investigated in relation to urbanisation than the number of offspring raised per breeding attempt. One important component of an individual's long-term fitness can be the benefits from fertilizations outside their pair bond, called "extra-pair fertilizations", which can increase the number of offspring for males and the fertility or genetic quality of offspring for females (Petrie & Kempenaers 1998). Several factors can potentially drive the extra-pair behaviour e.g. breeding density, inbreeding, territory quality), and most of these factors vary with urbanization. According to my knowledge, there is no information *whether individuals in urban and natural habitat types differ in their reproductive decisions about engaging in extra-pair copulations or not*, but investigating this aspect of reproductive success would deepen our knowledge about comparing lifetime reproductive success of individuals living in urban and natural habitats.

1.2. Sex ratios in different life stages

Both fitness of individuals and the long-term success of a population are influenced by the sex ratio (usually defined as the proportion of males) of the population, which may affect ecology and evolutionary traits as well as the life and behavioral characteristics of individuals (Székely, Weissing & Komdeur 2014b). The ratios of males to females can be investigated at conception, at birth, at independence and during adult life (termed primary, secondary, juvenile and adult sex ratio, respectively). The sex ratio of populations is often assumed to be balanced, partly due to Fisher's sex allocation theory (Fisher 1930), which states that only at 1:1 will the population sex ratio be stable and the expected success of males and females equal. It is because the rarer sex always has an advantage and selection favours parents that produce offspring of the rarer sex, but then the proportion of the rarer sex will increase, returning the population to 1:1 sex ratio. Even if the sex ratio deviates from 1:1 it will evolve back to that point (Fisher 1930). The above theory assumes that the cost of producing male and female offspring is equal, but that is not true for several species. In practice, sex ratios are often unbalanced, both in the younger developmental phases (Clutton-Brock & Iason 1986) and in adulthood (Donald 2007).

Several studies and even more hypotheses exist about the potential drivers of sex ratios in different life stages. The adult sex ratio (ASR) of a population is a fundamental demographic trait of individuals' social environment. Various ecological, life history and

demographic processes modulate the transition from sex ratios of earlier ages to ASR (Székely *et al.* 2014b). Unbalanced ASR can affect behavioural, demographic, and life-history characteristics in wild populations (Le Galliard *et al.* 2005; Clutton-Brock 2016). The number of potential partners can have a significant impact on mating opportunities, intra- and intersexual competition, and ultimately on the reproductive success (Székely *et al.* 2014b). The ecological and evolutionary implications of unbalanced sex ratios are significant as ASR bias can cause a decrease in population size (Le Galliard *et al.* 2005) and may influence the evolution of sex roles (Liker, Freckleton & Székely 2013). Multiple factors may lead to unbalanced ASR, including sex-specific mortality rates in early life stages and in adulthood, sex differences in growth, dispersal patterns, maturation or aging and unbalanced primary and/or juvenile sex ratios (Donald 2007; Lovich, Gibbons & Agha 2014; Székely *et al.* 2014a).

Unbalanced offspring sex ratio is predicted, on the one hand, when within-group interactions among relatives have a differential effect on the fitness of males and females (Hamilton 1967). This interaction can occur either competitively or cooperatively. If the dispersal of sons and daughters differ, then parents should invest less in the non-dispersing sex, because individuals of this sex compete with parents and each other (local resource competition hypothesis). This theory is supported frequently for birds and mammals with biased sex ratios, for example, birth sex ratio is biased towards the dispersing sex in primates across 102 species (Silk & Brown 2008). Relatives can also cooperate instead of competing, e.g. if one sex is more likely to remain and help the parents, then the offspring sex ratio should bias towards the helping sex because it is beneficial to produce an offspring which helps rearing the future offspring and increase the lifetime reproductive success of the parents (local resource enhancement hypothesis). This theory is supported for cooperatively breeding birds and mammals, either when males are more likely to help like in African wild dogs (*Lycaon pictus*) and red-cockaded woodpeckers (*Leuconotopicus borealis*), or when females are more likely to help like in Seychelles warblers (*Acrocephalus sechellensis*) (Davies, Krebs & West 2012).

On the other hand, differential allocation of sexes can be advantageous not only when relatives interact, but also when the environment is variable and different environments affect males and females differently. Robert Trivers and Dan Willard (1973) suggest that environmental conditions and maternal quality can cause differences in the fitness of sons and daughters, thus parents should adjust the sex of offspring in response to which sex is beneficial under given circumstances (Trivers & Willard 1973).

Suppose that good quality environments and/or mothers in good condition have more resources thus can produce better quality offspring, and better quality offspring become more successful adults. When competition of one sex is intense for mates then this sex gains greater benefits from being high quality adults, so mothers in good condition and/or in better breeding environment should allocate more into this more competitive sex (usually males in bird and mammal species), while low quality mothers should invest more to the other sex. This hypothesis was supported in several research (summed in e.g. Hasselquist & Kempenaers 2002; Szász, Kiss & Rosivall 2012).

Beside differential investment into producing the two sexes, parents can invest differentially into rearing male and female offspring depending on the quality of their mate (Szász, Garamszegi & Rosivall 2019). For example, eastern bluebird (*Sialis sialis*) parents fed their sons more frequently than their daughters when they had a high-quality ornamented mate (Ligon & Hill 2010), while the aggressive behaviour of male parents predicted the proportion of sons in their brood in great tits (*Parus major*) (Radford & Blakey 2000) and in collared flycatchers (*Ficedula albicollis*) (Szász *et al.* 2014). Moreover, sons and daughters may have different cost to produce or rear successfully, because the sexes can react differently to environmental conditions during their development, and unbalanced brood sex ratio (BSR) can emerge via different male and female embryo mortality (Göth & Booth 2005), or sex-differential sensitivity after birth to certain environmental conditions such as food availability (Kilner 1998; Martins 2004), hatching asynchrony (Bowers, Sakaluk & Thompson 2011) or number of offspring in a brood (Rosivall *et al.* 2010). Similarly, the sex with higher growth rate may be more sensitive during early development and/or may be more costly for parents to raise. Therefore, sexual size dimorphism (SSD, differences in body size between males and females) can have important implications for the evolution of sex ratios, because size differences may result in differential costs of rearing sons and daughters, which in turn may skew the sex ratios towards the smaller sex at the population level (Benito & González-Solís 2007; Piedrahita *et al.* 2014). Differential costs of producing male and female offspring may originate even at the stage of sperm or ova production in species with genetic sex determination (GSD) where the sex of an offspring is determined at the moment of fertilization because of some sex determinant genes usually located at specific chromosomes. In birds, females are heterogametic as they have two different sex chromosomes, a large Z and a smaller W, and there is evidence for primary sex ratio adjustment with several potential mechanisms in species from half of the avian orders

(reviewed in Pike & Petrie 2003), which suggests that this phenomenon is quite widespread in birds. In mammals, where XY sex chromosome system exists, males are heterogametic with the sex of the offspring determined by the inheritance of either an X or a Y chromosome from their father, and there is some evidence that producing X- and Y- containing sperm cells may have different costs (Edwards & Cameron 2014). By investigating mostly vertebrates with GSD system, recent knowledge is that organisms are often manipulating the sex of their offspring in several ways to increase their fitness (Pike & Petrie 2003; West 2009), this is called sex allocation strategies. *It is possible then, that environmental circumstances such as weather conditions or suboptimal habitat types like cities are such factors, that birds may try to increase their reproductive success by unequal investment into sons and daughters. Alternatively, male and female offspring may also differ in their sensitivity to extreme weather events or urban habitat.*

In addition to sex allocation, sex chromosomes may also affect the sex ratio of populations. *Theoretically, the genetic sex determination system can affect the sex ratio of a species. According to the “unguarded sex chromosomes hypothesis” (Trivers 1972), the heterogametic sex would be underrepresented in a population, because it is unguarded against the recessive deleterious mutations located in the X or Z chromosomes. The heterogametic sex has only one copy of its sex-chromosome genes, while in the homogametic sex (e.g. female mammals and male birds) such a mutation can be compensated by the well-functioning gene in the other copy of the sex chromosome. This hypothesis has not been tested with interspecific comparative research.*

1.3. Thesis objectives

During my doctoral studies, I focused on two actual and globally relevant topics. First, I tested how two factors related to anthropogenic environmental change, weather variability and habitat urbanisation, affect reproductive behaviour, breeding success, and brood sex ratios in birds. I studied these questions in two passerine species, the house sparrow (*Passer domesticus*) and the great tit (*Parus major*). Second, I investigated the causes of adult sex ratio variability of vertebrate populations, a fundamental yet under-studied element of the social environment of individuals.

Specifically, I aimed to answer the following research questions:

1. How does extreme weather influence the reproductive success and offspring sex ratio of house sparrows (*Passer domesticus*) and great tits (*Parus major*)?
2. Do the effects of extreme weather on reproductive success of great tits differ between urban and forest habitats?
3. Are there any differences in brood sex ratios and in the frequency of extra-pair fertilizations between great tit populations breeding in urban and forest habitats?
4. Is there a relationship between the type of genetic sex-determination system and the adult sex ratio in vertebrate species?

In this thesis, I address questions 1-3 in chapters 3-6 using data from two long-term field projects. I address question 4 in chapter 7 with a phylogenetic comparative study using literature data on 344 tetrapod species (mammals, birds, reptiles and amphibians).

2. General methods

2.1. Field methods (for studies in chapters 3-6)

All of the bird study sites are located in Hungary, which is a country situated in Central Europe about halfway between the Equator and the North Pole. It is in the temperate climate zone, but its climate is very variable, mainly because Hungary is affected by three other climatic zones: the oceanic climate with less varying temperature and more evenly dispersed precipitation; the continental climate with more extreme temperatures and relatively moderate precipitation; also, a Mediterranean effect with dry weather in summer, and wet one in winter. Due to these reasons great differences can occur in the weather of the country, despite of its relatively low altitudes and relatively small area (www.met.hu/en/eghajlat, Hungarian Meteorological Service, 2019). The annual precipitation amount in Hungary is 500–750 mm, the most precipitation usually falls from May to July (58-71 mm per month) whereas the warmest period of a year is usually between late July and early August. The monthly mean temperatures from March to July are between 5 °C and 25 °C. Our study sites are located in a moderately cool and moderately dry region of the country.

Studies on house sparrows. The reproduction of house sparrows was studied in a nest box-breeding population in the Kittenberger Zoological Garden of Veszprém, Hungary (N 47°05'32", E 17°53'44") between 2004 and 2011. House sparrow is a slightly size dimorphic passerine as males are about 2% larger than females (Anderson 2006). They can rear 1-3 broods per breeding season from the end of March to August, thus each nest box was checked at least twice a week during this interval, and the number of eggs or nestlings was recorded. Nestlings were ringed before fledging at the age of 10 days, using an individual combination of one aluminium and three plastic rings, with two rings on each tarsus. Upon ringing, we measured each nestling's body weight (± 0.1 g) by a spring balance and the length of the left tarsus (± 0.1 mm) by a vernier caliper. From 2005, small blood samples were taken from a subset of offspring (i.e. all offspring from a brood alive when ringing occurred, but only from a subset of broods) upon ringing by brachial venipuncture, and stored in Queen's lysis buffer at room temperature until the laboratory procedures.

Studies on great tits. Nest-box colonies were set up for monitoring great tits from 2012 in urban (city of Veszprém 47°05'17"N, 17°54'29"E), and natural habitats (forests near Vilma-puszta 47°05'06.7"N, 17°51'51.4"E and Szentgál 47°06'39"N, 17°41'17"E), and additionally from 2013 in an other urban site (Balatonfüred 46°57'30"N, 17°53'34"E). Thus, we have two urban and two forest study sites (Figure 2.1). Urban nest-boxes are located mostly in public parks, university campuses and a cemetery, where vegetation contains both native and introduced plant species. Forest study sites are located in deciduous woodlands, characterized by beech *Fagus sylvatica* and hornbeam *Carpinus betulus* (in Szentgál) or downy oak *Quercus cerris* and South European flowering ash *Fraxinus ornus* (in Vilma-puszta).

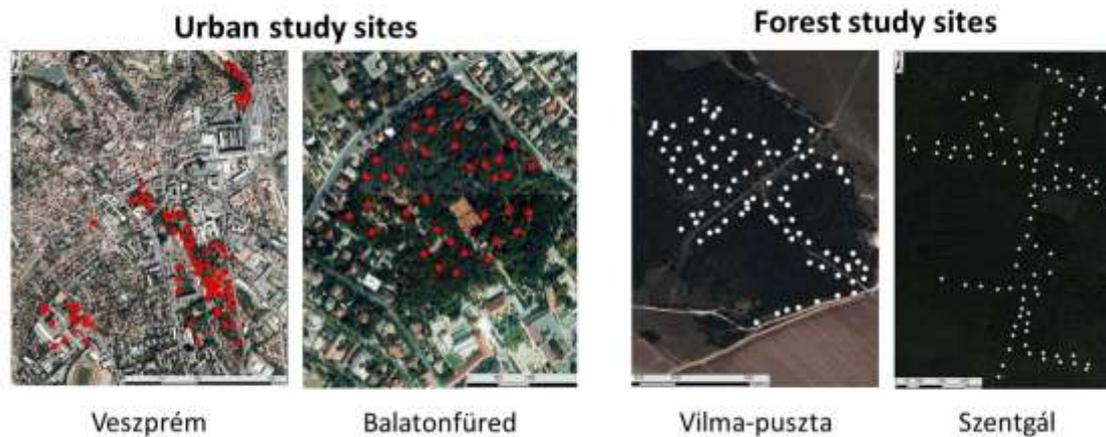


Figure 2.1. Study sites for great tit studies with the location of nest-boxes (dots).

Great tits usually rear maximum two broods per breeding season, thus, we recorded the number of eggs and nestlings in the nestboxes every 3-4 days from March to the end of July. In each study site, a clutch was regarded as first brood if it was initiated before the date of the first egg laid in the earliest second clutch at that site by an individually identifiable (i.e. colour-ringed) female that had a successful first breeding (i.e. fledged at least one young) in that year. We captured parent birds using a nest-box trap 6-15 days after their first nestling had hatched. We determined parents' sex based on their plumage characteristics, measured their tarsus length with a Vernier caliper, their wing length with a wing ruler, their body mass with a Pesola spring balance and ringed each bird with a unique combination of a numbered metal ring and three plastic colour rings. Breeding adults ringed on previous occasions were identified by observing their ring combination from recordings made by a small, concealed camera put on the nest boxes in the chick-rearing period (Seress et al. 2017). On these video samples we considered a colour ringed

individual to be a parent bird if it was recorded to enter the nest box with food at least once. When the nestlings reached the age of 14-16 days we measured and ringed them using the same methods as with their parents. From all captured adults and from a subset of whole broods (i.e. all offspring in selected families), we took a small amount of blood sample (ca. 25 μ l) from the brachial vein into 500 μ l Queen's lysis solution or 96 % ethanol. From 2013, we also collected the eggs that did not hatch five days after the first nestling had hatched, as well as tissue samples from the nestlings that were found dead before blood sampling, and stored them in 96 % ethanol. All tissue samples were stored at 4 °C until laboratory work.

In March 2013, we installed a WH 2080 weather station (Ambient, LLC, AZ, USA) near each of our study sites, which record temperature, humidity, air pressure, precipitation and wind speed and direction. We also put one Voltcraft DL101T temperature and humidity data logger (Conrad Electronic SE, Germany) to an empty nestbox within each of our four nestbox colonies. Thus, we have hourly weather data for all of our four study sites.

All procedures applied during all of the research presented here were in accordance with the guidelines for animal care outlined by ASAB/ABS (www.asab.org) and Hungarian laws. We have all the required permissions for capturing, measuring of the birds, monitoring their breeding and taking blood samples from the Balaton Upland National Park (permission number: 9135-2/2004, 2255/2008) and from the Government Office of Veszprém County, Nature Conservation Division (former Middle Transdanubian Inspectorate for Environmental Protection, Natural Protection and Water Management; permission number: 31559/2011). Both avian species in our studies are protected in Hungary and listed in the "Least Concern" category of the International Union for Conservation of Nature's Red List of Threatened Species, although the house sparrow has been suffering moderate population declines in Hungary since 1999 (Szép *et al.* 2012) and severe declines in several other countries (De Laet & Summers-Smith 2007).

2.2 Laboratory methods (for studies in chapters 3, 5 & 6)

Laboratory work was conducted at the molecular laboratory of the Department of Ecology, Institute of Biology, University of Veterinary Sciences, Budapest. For the study on brood sex ratio of house sparrows (chapter 3), samples were analysed in 2011. DNA

was extracted using standard phenol-chloroform extraction (Gemmell & Akayima 1996). Sex was determined by PCR amplification of the *CHDI-W* and *CHDI-Z* genes, using the 2550F/2718R primer pair (Fridolfsson & Ellegren 1999). This primer pair produced ambiguous results with some samples (n=45), so in these cases we repeated sexing using the P2/P8 primers (Griffiths *et al.* 1998) to clarify the results. PCR reactions (with both primer pairs) were performed using the conditions as described by the authors publishing the primers (Griffiths *et al.* 1998; Fridolfsson & Ellegren 1999).

For studies on great tits (chapter 5 & 6), samples were analysed in autumn 2014. DNA was extracted using silica membrane isolation kits (GeneJET, Genomic DNA Purification Kit) following the manufacturer's protocol (Thermo Scientific™) for both blood and other tissue samples. We used blood samples of ringed individuals and we also collected small tissue samples from dead chicks found in the nest before fledging and from embryos found in the unhatched eggs. We used only those unhatched eggs which contained a developed, identifiable embryo. We treated unhatched eggs with no embryo as infertile eggs. Molecular sexing (for chapter 5) was performed using the primer pairs P2/P8 as described by the authors publishing the primers (Griffiths *et al.* 1998). In a subset of samples (n=170 taken in 2014), direct PCR method was used to reduce the cost of laboratory work. Direct PCR process was done without DNA extraction, using the following PCR profile: initial denaturation at 98°C for 3 minutes, followed by 40 cycles of 98 °C for 6 sec, 56 °C for 15 sec, 72 °C for 20 sec. Direct PCR reactions were made in 20 µl volumes containing 1 µl of diluted blood template, 10 µl of Phire Tissue Direct PCR Master Mix (Thermo Fisher Scientific, USA) and 10 pmol of the respective primer(s). PCR products were evaluated by agarose gel-electrophoresis by two independent observers.

For a subset of families, we conducted multilocus genotyping with 5 loci to examine extra-pair paternity (for chapter 6). We used available microsatellite markers (Table 2.2.1), where forward primers were labelled with fluorescent dyes (Fam-6, NED, PET, or HEX) on the 5' end; reverse primers contained a GTTT pigtail sequence on their 5' end. PCR reactions were performed in 20 µl volumes, containing 10-30 ng of total genomic DNA template, 1 U of DreamTaq polymerase (Fermentas), 1 × DreamTaq PCR buffer (Fermentas), 1.5 mM MgCl₂, 10 pmol dNTPs (Fermentas) and 10 pmol of the respective primer(s). PCR profiles were the following for all loci: initial denaturation at 95°C for 2 minutes, followed by 39 cycles of 95 °C for 30 sec, 57 °C for 45 sec and 72 °C for 45 sec, concluded by a final extension step at 72°C for 7 min. In a subset of samples (n=23

individuals out of total 1010) with ambiguous results based on the 5 loci, we used 3 additional loci with the same protocol as described (Table 2.2.1). Fluorescent PCR products were scanned by capillary electrophoresis on an Abi 3130 Genetic Analyser (Thermo Fisher Scientific); alleles were identified and scored with PEAKSCANNER software (Thermo Fisher Scientific) by two independent, experienced researchers who were blind to the identity of individuals.

Table 2.2.1. Observed allele diversity, probability of identity (PI), probability of exclusion with both parents known (PE2) and with only one parent known (PE1), and GenBank accession number of the microsatellite loci used in the study

Locus	No. of alleles	PI	PE2	PE1	GenBank
PmaGAn27	22	0.011	0.846	0.732	AY260532
PmaTAGAn89	14	0.035	0.719	0.558	HQ263126
PmaTGAn33	16	0.023	0.773	0.630	AY260539
PmaTGAn54	32	0.031	0.733	0.576	HQ263130
PmaTGAn59	20	0.016	0.814	0.686	HQ263131
5 loci combined	104	4.26×10^{-9}	>0.999	0.994	
PmaCAN1*	11	0.036	0.715	0.554	AY260530
PmaTAGAn73*	9	0.054	0.653	0.480	HQ263122
PmaTAGAn78*	17	0.014	0.823	0.700	HQ263123
8 loci combined*	141	1.43×10^{-12}	>0.999	0.999	

The data are based on 159 adults for the first five loci and 23 adults for the three loci marked with an asterisk.

2.3. Phylogenetic comparative methods (for study in chapter 7)

We collected data for four major clades of tetrapods (amphibians, reptiles, birds and mammals) to assess whether ASRs (proportion of males in the adult population) differ between taxa with XY and ZW sex determination systems. Data on ASR in amphibians and reptiles were collected from literature published by December 2013, by searching in Google Scholar and Web of Science with the key words "sex ratio" and "reptile" or "amphibian" or the scientific names of species. We also used reviews to identify additional data sources (Jongepier 2011; Evans, Pyron & Wiens 2012). ASR data for mammals were obtained from a similar search finished in 2007 (Donald 2007), and we used avian ASR estimates from an existing data set (Supplementary Information of Liker, Freckleton & Székely 2014).

We specifically collected ASR data for amphibians and reptiles from studies that aimed to obtain representative estimates for the population composition and thus provide

reliable sex ratio data (Arendt, Reznick & López-Sepulcre 2014). These include either long-term demographic studies applying mark-recapture or sacrificing methods (i.e. each individual was counted only once) with similar capture probabilities for the sexes, or total population counts. We categorized the genetic sex-determination (GSD) systems of the species from published sources either as male-heterogametic (XY) or female-heterogametic (ZW). Our final dataset comprises data on 39 amphibian species and 67 reptile species (in total $n = 229$ ASR records from different populations), 187 bird species and 51 mammalian species (a total of 344 species).

We assembled a large composite phylogeny for all the species we had data about. Phylogenies were taken from recent molecular studies (see details in chapter 7 and Figure 7.1). To test for differences in ASRs between XY and ZW taxa, we used phylogenetic generalized least squares models (PGLS) (Pagel 1998; Freckleton, Harvey & Pagel 2002), and we used Pagel's discrete method (PDM) (Pagel 1994) to test whether XY and ZW systems are evolutionarily associated with female-biased and male-biased sex ratios, respectively. These methods are adequate to control for phylogenetic dependencies between species. Detailed methods and additional analyses validating our data collection can be found in chapter 7 and Appendix A/3, respectively.

3. Effects of extreme weather on reproductive success and brood sex ratio of house sparrows (*Passer domesticus*)¹

Introduction

Global average temperature is increasing on the Earth, and this process has been getting faster in the last 50 years (Parmesan 2006). Besides climate warming, increases in the frequency and magnitude of meteorological extremities are also expected (Jentsch & Beierkuhnlein 2008), as the observed and projected data predict more hot days, more extreme rainfalls and longer droughts for most regions of the Earth (Field *et al.* 2012). The largest anomalies are measured in summer when most biological productivity occurs, so this is probably the season when climate change will have its greatest impact on ecosystems (Hansen, Sato & Ruedy 2012).

Wildlife species' range, habitat, phenology, demographic and morphological traits can change in response to climate warming (Crick 2004; Yom-tov *et al.* 2006; Kovács *et al.* 2010; Lavergne *et al.* 2010), and the magnitude of these responses depends on several ecological and life-history characteristics of the species (Végvári *et al.* 2010). Differential responses by different species (e.g. predator and prey) may lead to phenological mismatches which can alter the rates of reproduction and survival, causing decline in some populations and increase in others (Shultz *et al.* 2005; Miller-Rushing *et al.* 2010; Hegyi & Nagy 2012). For example, Møller *et al.* (Møller *et al.* 2008) have found in a comparative study that birds that did not respond to recent climate change by shifting their spring migration phenology have declining breeding populations, whereas species that advanced their timing of migration have stable or increasing populations in Europe. This finding is supported by a similar study on the phenology of egg-laying (Salido *et al.* 2012). Thus, climate change may have crucial fitness consequences in animal populations. Understanding and predicting these effects requires detailed knowledge about the effects of different aspects of weather on the biota.

Traditionally, meteorological conditions were included into the studies of reproductive success mostly as background variables (Elkins 2004), and the effects of weather *per se* on individuals or populations have been rarely studied up to recently

¹ This chapter is based on the research article **Pipoly, I., Bókony, V., Seress, G., Szabó, K., Liker, A. (2013):** Effects of extreme weather on reproductive success in a temperate-breeding songbird, *PLoS ONE*, 8: (11), 1-11

(McDonald, Olsen & Cockburn 2004; Smith *et al.* 2010; Knape & de Valpine 2011; Vincze *et al.* 2013). Despite the recognized need for predicting the effects of increasing weather extremities (Katz & Brown 1992; Jentsch & Beierkuhnlein 2008), such effects are investigated mostly in connection with human health (Deschenes & Moretti 2007; Gasparri & Armstrong 2011; Field *et al.* 2012), whereas we know very little about the ability of animals to cope with such conditions (Møller 2011). Therefore, beyond the long-term phenological monitoring of populations (Crick & Sparks 2006; Csörgő, Harnos & Kovács 2009), reproductive behaviour and fitness should be studied in relation to weather extremities to understand how meteorological events get translated into responses at the level of individuals and populations.

In birds, prevailing weather can affect the main components of reproduction such as hatching success and fledging success in several ways. Low temperatures may make it difficult to maintain the optimal temperature of eggs (e.g. when parents have to interrupt incubation), and young nestlings that lack own thermoregulation are also very vulnerable to chilling (Elkins 2004). Access to food may also be related to weather, either because prey may be less available during certain meteorological circumstances (Taylor 1963), or because the ability of parents to collect food may be affected (Radford *et al.* 2001; Pipoly, Bókony & Liker 2011). Besides the components of reproductive success, weather may also influence the sex ratio of offspring. One sex can be more sensitive to environmental conditions than the other (Kilner 1998; Rosivall *et al.* 2010), thus extreme or unfavorable weather may affect sons and daughters differently during their ontogeny; however, this phenomenon is yet little studied (Torres & Drummond 1999; Weatherhead 2005). Furthermore, offspring sex ratio can also be altered by differential parental investment, e.g. parents in some species may benefit by preferring more sons under favourable conditions (Ligon & Hill 2010; Dijkstra *et al.* 2010).

In this study our goal was to understand the effects of prevailing weather and extreme meteorological events on the breeding biology of a hole-nesting sedentary bird species, the house sparrow (*Passer domesticus*). Specifically, we investigated how local temperature and precipitation during incubation and nestling development influence hatching success, body size, fledging success and sex ratio of nestlings. We focused on two aspects of weather: the overall conditions during each period and the frequency of extremities.

Methods

Data collection

Field methods are described in section 2.1. Date of laying was either ascertained during laying, since house sparrows lay one egg per day (Anderson 2006), or estimated as 11 days minus hatching date if the clutch was found complete (average length of incubation period was 10.58 ± 0.08 (SE), $n = 230$ clutches). Date of hatching was either ascertained by checking the nest on consecutive days or estimated from the developmental state of nestlings when hatching had occurred in the inter-monitoring interval. As house sparrows have high nest-box fidelity, we considered the parents as the same individuals for consecutive broods in the same nest-box during a single breeding season (Anderson 2006), because we had no detailed information about the identity of parents for the majority of broods. When a nestbox was occupied in more than one year throughout the study, we randomly selected data from only one year to avoid pseudoreplication among years. We considered the ringed nestlings as a proxy for successful fledglings, because we did not disturb the nest-boxes after ringing for about a week to prevent premature fledging. There were 317 clutches where at least one nestling hatched; and 736 nestlings reached the pre-fledging age in 227 broods (Appendix, Table A/1.1).

Offspring sexing

Information about the sex of house sparrow offspring originated from two sources. On the one hand, sex was known from recapture and/or resighting data from the study area for $n=92$ individuals (51 males, 41 females) that hatched in the studied broods. On the other hand, the sex of 193 nestlings (89 males, 104 females) was determined by molecular genetic method (section 2.2). Due to financial constraints we had to limit genetic analyses to 20 broods per year chosen randomly from all broods hatched between 2005-2007, summing up to 60 broods and 236 nestlings. We always sexed whole broods, i.e. all nestlings being alive at the age of ringing in a given nest was sexed (however, we had no DNA samples from unhatched embryos or nestlings that died before sampling; we had 20 broods in which all the laid eggs hatched and were blood-sampled). To verify the molecular results, we additionally analysed the blood samples of $n=39$ individuals whose sex was known from resighting and/or recapture data. Sex determined by molecular analysis agreed with sex registered during resightings and/or recaptures in all but one case (in this latter case, we suspect that the resighting record was mistaken).

Meteorological variables

Throughout the study, a meteorological station (HW WS 2350) about 2800 meters from the study area (N 47°10'16", E 17°93'14") collected data on daily minimum and maximum temperatures (°C) and daily amount of precipitation (mm). These data were used to create meteorological variables that characterize weather conditions for two periods for each nest: the incubation period of clutches (from the day of laying the penultimate egg to the day of first hatching), and the nestling period (from the day of first hatching to the day preceding the day of ringing and measuring). First we calculated two variables that represent the overall weather conditions during each period: daily mean temperature as the mean of daily minimum and maximum temperatures averaged over the period, and total amount of precipitation during the period. Then we calculated four variables to express the frequency or extent of extreme conditions during each period. 1) The number of hot days was defined as the number of days when daily maximum temperature was higher than 30.9°C, the 90th percentile of our daily maximum temperature data in April-August 2005-2010. This value corresponds well with the definition used in human meteorology, i.e. days with >30°C maximum temperature are considered heat days ("Hungarian Meteorological Service" 2013). We also validated our definitions of extremities using a 100-years database measured in 1901-2000 ca. 100 km from our study site (Hungarian Meteorological Service, Budapest); the 90% percentile of daily maximum temperature in this dataset was 30.8°C, indicating that hot days by our definition were indeed rare extremities during the last century in our region. 2) The number of cold days was defined as the number of days when daily maximum temperature was below 15.9°C, the 10th percentile of our April-August data (15.3°C in the 100-years dataset). 3) The number of heavy rain days was defined as the number of days when the amount of daily precipitation was higher than 10 mm, the 90th percentile of our data (11.3 mm in the 100-year dataset; human meteorology also uses the 10 mm threshold) (Hungarian Meteorological Service 2013). Finally, 4) the number of dry days was defined as the maximum number of consecutive days when no precipitation was recorded till the end of the incubation or nestling period; this variable expresses the length of uninterrupted dry period preceding hatching or fledging, respectively. For example, if the last rainfall during the period occurred 5 days before the end of the period, then the number of dry days was 4, irrespective of the number of rainy days before the last rainy

day. The length of continuous dry periods as defined above varied between 1-26 days, thus the entire incubation or chick rearing could coincide with a period without any rain.

Statistical analyses

We calculated the following variables to quantify components of reproductive success. Hatching success was the percentage of hatched eggs in those nests where at least one chick hatched. Fledging success was the percentage of hatched young that were alive at the age of ringing in those nests where at least one nestling reached that age. We excluded nests in which no chick hatched or no chick reached the age of ringing from the calculation of hatching and fledging success, respectively, because the period for which the meteorological variables should be calculated was not comparable with (i.e. was much shorter than) the incubation and nestling periods of successful nests. For each brood, we calculated the mean body mass and mean tarsus length of nestlings to avoid pseudo-replication because the values of siblings cannot be treated as non-independent data points. Date was measured as the number of days from 1st of January within each study year until the day of hatching of the first chick in each brood. Period length was defined as the length of incubation period in the analyses of hatching success, and as the length of nestling period in the analyses of fledging success (see above). Nestling periods (i.e. age of nestlings) and brood size were additionally included in the analyses of nestlings' size. Nestling periods ranged from 7 to 15 in our analyses, and body mass changed linearly with age according to our data. Brood size was the number of nestlings in a brood at the age of ringing.

We used structural equation modelling (SEM) to investigate the correlations between reproductive success and weather conditions. SEM is a multivariate statistical method particularly useful for decomposing the covariation within complex sets of multi-colinear variables (Grace *et al.* 2010; Dingemanse, Dochtermann & Wright 2010). We fitted structural equation models by the method of maximum likelihood using AMOS 20.0 (Arbuckle 2011). Because the error distribution of our data was not normal, the 95% confidence intervals of path coefficients were estimated by bootstrapping, with 9000 bootstrap samples for each model (Dingemanse *et al.* 2010). For each of the four measures of reproductive success (dependent variables), we constructed a set of nested *a priori* models (Tables A/1.2-6). In each model set, the full model estimated reproductive success as function of both the two overall and four extreme meteorological variables

(Figure 3.1). Further candidate models contained various plausible combinations of these six weather variables and a „null model” with no weather effects (Table A/1.2-6).

All models of hatching success contained the effect of the number of cold days on period length, because cold weather can lengthen the incubation period (Strausberger 1998; Hepp, Kennamer & Johnson 2006). This path was not included for the rest of the dependent variables (fledging success and nestlings' size), because period length was set by researchers at ca. 10 days in these cases by measuring the nestlings around 10 days of age. For hatching success, we constructed additional models including the interaction between the number of cold days and the length of incubation period (Table A/1.2), because we expected that the effect of cold days may depend on whether or not parents adjust incubation length to the cold (Reid, Monaghan & Ruxton 2000, 2002). All models in each model set included the direct effect of date on reproductive success, since date may influence breeding not only via its impact on weather but also through other seasonal changes, such as the seasonal decline of food availability or offspring value and thereby parental effort (Hoi, Vaclav & Slobodova 2003; Dawson 2008). As potential confounding variables, all models included the effect of period length (incubation period or nestling period) on reproductive success, and models of nestlings' body mass and tarsus length also contained the effect of brood size (e.g. sibling competition).

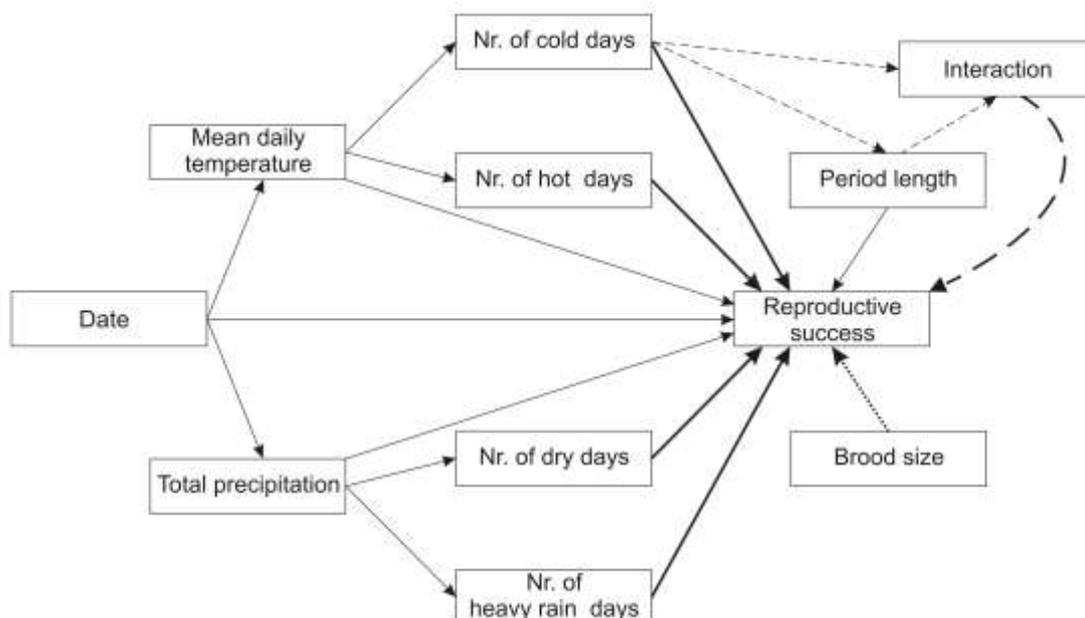


Figure 3.1. Model structure in SEM analyses. Thin lines stand for effects included in all models, thick lines for relationships that varied within model sets, dashed lines for paths contained only in the model set of hatching success, and the dotted line for the effect of brood size in model sets of nestlings' body mass and tarsus length.

Each model set was evaluated using the information-theoretic approach, comparing the candidate models by their Akaike's information criterion (AIC) value (Burnham & Anderson 2002). For each model we calculated its AIC difference from the „best model” (i.e. the model with the lowest AIC-value in the model set) and its Akaike weight which estimates the probability that the model is actually the best model in the model set. Then we used the model-averaging approach to calculate model-averaged parameter estimates and unconditional variances for each variable based on the whole model set (Burnham & Anderson 2002). All variables were z-transformed prior to the analyses, as recommended for SEM analyses (Dingemanse *et al.* 2010), thereby the values of parameter estimates can be interpreted as standardized effect sizes, i.e. the amount of change in units of SD in the dependent variable's value in response to 1 SD increase in the predictor's value. According to Cohen's rule of thumb, effects above 0.2, 0.5 and 0.8 are considered small, medium and large, respectively (Cohen 1988), whereas mean effect size ranges 0.22 - 1.7 in ecological studies (Møller & Jennions 2002). Thus, we defined important effects as paths with $>|0.2|$ parameter estimates, and/or 95% confidence intervals $>|0.2|$ at one side and not including zero (or including zero but very close to it) on the other side; note that confidence intervals including zero do not necessarily mean the lack of effect (Burnham & Anderson 2002; Nakagawa & Cuthill 2007).

Additionally, we investigated whether the relationship between nestlings' body size and weather differed between male and female offspring by using multigroup analysis (Dingemanse *et al.* 2010; Arbuckle 2011), which compares the variance–covariance matrices of SEM models between groups. We ran the full model shown in Figure 3.1 for both body mass and tarsus length in two ways: first constraining the parameter estimates of paths from meteorological variables towards the dependent variable to have the same value for both sexes, then allowing them to differ between sexes. The fit of these two models were compared by χ^2 tests based on minimum discrepancy (\hat{C}_{\min}) (Arbuckle 2011).

Although weather or reproductive success may change non-linearly over the season, the quadratic effect of date was not included into our models because graphs indicated that the seasonal variation of both temperature and precipitation can be sufficiently described by linear models in our study period. Any potential quadratic effect of weather on reproductive success was modeled by the simultaneous inclusion of overall and extreme meteorological variables. Although consecutive broods in the same nest box (presumably by the same pair) are repeated measures, we did not include random effects

into our models because the current implementations of SEM cannot handle random factors. To evaluate the importance of repeated measures, we built linear mixed-effect models for each dependent variable and compared pairs of models with and without the random effect (i.e. nestbox ID) using likelihood ratio tests in R (R Core Team 2014). We found that models without the random effect fit our data similarly well as models containing the random effect ($\Delta AIC < 2$, $p > 0.156$ in all cases). The non-independence of within-brood siblings' data was handled by using their averages per brood (see above).

Results

Overall, the effects of weather variables on reproductive success were small, as effect sizes ranged between 0 and 0.43 (absolute values; Table 3.1). Nevertheless, some confidence intervals included moderate or even strong effects of weather on hatching success and nestling morphology (Table 3.1). Notably, the range of weather effects were comparable in magnitude to those of other ecologically relevant predictors of breeding success, i.e. date, length of incubation period, nestling age and brood size (0.03-0.41; Table 3.1).

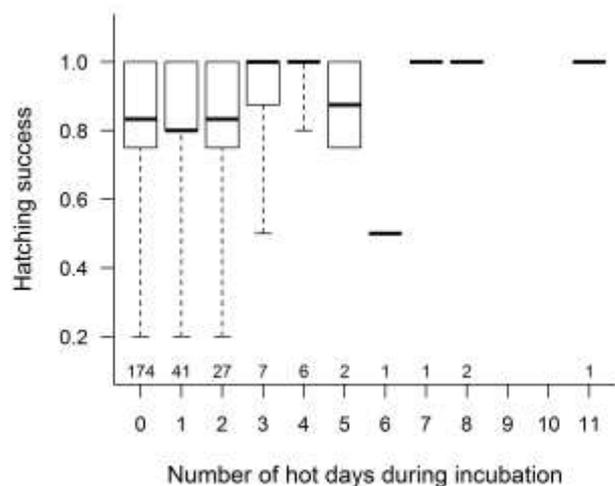


Figure 3.2. Relationship of hatching success with the number of hot days during incubation. Box plots show the median (thick line), interquartile range (box) and the range of data (whiskers); sample sizes are shown below each box.

For hatching success, two important meteorological effects emerged (Table 3.1, Table A/1.1). A greater proportion of eggs hatched when there were more extremely hot days (Figure 3.2) and fewer extremely cold days during incubation. However, the latter effect held only for clutches with short incubation periods (Figure 3.3, see regression plane edge indicated by white arrow). More cold days were associated with increased incubation period length (Figure 3.3, bottom grid and grey dots), and longer incubation in cold periods was correlated with higher hatching success (Figure 3.3, light-grey arrow), but prolonged incubation during non-cold periods was associated with reduced hatching

success (Figure 3.3, dark-grey arrow), leading to a positive relationship between the number of cold days and hatching success for long incubation periods (Figure 3.3, black arrow). For fledging success, all meteorological variables had negligible effects (Table 3.1, Table A/1.2). Longer nestling periods (i.e. later ringing of nestlings) were associated with lower fledging success (Table 3.1).

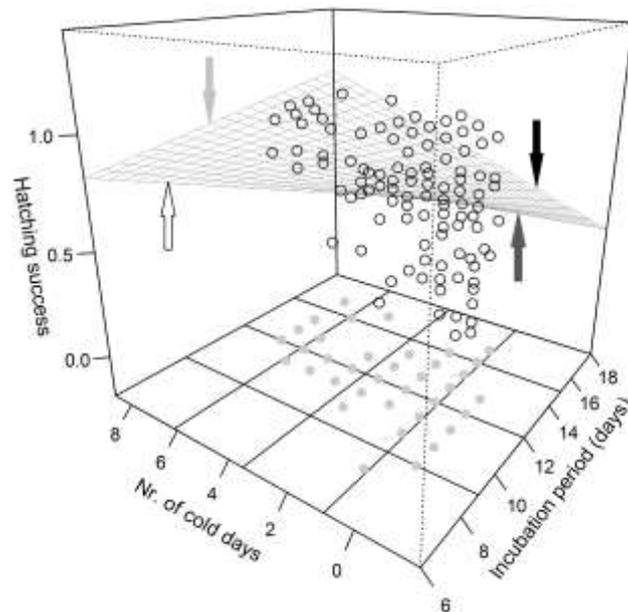


Figure 3.3. Relationship of hatching success with the number of cold days and length of incubation period. The warped regression plane was fitted from a linear regression to illustrate the interacting effects of the two predictors on hatching success. Open circles are the data points in 3D space defined by the three variables. Grey dots on the bottom grid of the graph show the relationship between the number of cold days and length of incubation period. Arrows highlight the slopes of the relationships between hatching success and number of cold days when incubation is short (white) or long (black), and between hatching success and length of incubation when number of cold days is high (light-grey) or low (dark-grey).

Both body mass (Figure 3.4A, Table A/1.4) and tarsus length (Figure 3.4B, Table A/1.5) of nestlings at pre-fledging age were larger in periods with higher daily mean temperature whereas the frequency of hot days had a smaller opposing effect (Table 3.1). Furthermore, nestlings weighed more when there was a longer period without rain before fledging (Figure 3.5, Table 3.1). Additionally, nestlings that hatched later in the breeding season weighed less, those in bigger broods had longer tarsi, and older nestlings had larger body mass and tarsus length (Table 3.1).

1 **Table 3.1: Model-averaged parameter estimates [95% confidence intervals] for five measures of reproductive success as dependent variables.**
 2 A higher parameter value indicates higher effect size along the path in SEM. Paths highlighted in bold and italics show the effects of weather and non-
 3 weather variables on dependent variables, respectively. Effects considered as important are shown in blue (parameter estimates > |0.2|, and/or 95%
 4 confidence intervals > |0.2| at one side and not including zero (or including zero but very close to it) on the other side).

Path in SEM		Hatching success	Fledging success	Body mass	Tarsus length	Brood sex ratio
Daily mean temperature	→ Dependent variable	-0,06 [-0.27; 0.15]	0.01 [-0.15; 0.18]	0.43 [0.06; 0.81]	0.43 [0.07; 0.79]	-0.02 [-0.34; 0.30]
Total amount of precipitation	→ Dependent variable	-0.05 [-0.25; 0.14]	0.04 [-0.13; 0.21]	0.08 [-0.10; 0.26]	-0.01 [-0.16; 0.14]	0.00 [-0.28; 0.28]
Nr. of hot days	→ Dependent variable	0.14 [-0.03; 0.32]	0.03 [-0.06; 0.11]	-0.10 [-0.25; 0.05]	-0.11 [-0.25; 0.03]	-0.07 [-0.29; 0.14]
Nr. of cold days	→ Dependent variable	-0.20 [-0.51; 0.11]	-0.02 [-0.10; 0.06]	-0.01 [-0.09; 0.08]	0.04 [-0.06; 0.15]	0.02 [-0.12; 0.15]
Nr. of dry days	→ Dependent variable	0.01 [-0.06; 0.08]	0.03 [-0.05; 0.11]	0.23 [0.03; 0.42]	0.05 [-0.05; 0.15]	-0.07 [-0.27; 0.14]
Nr. of heavy rain days	→ Dependent variable	0.00 [-0.12; 0.13]	-0.04 [-0.15; 0.07]	-0.02 [-0.13; 0.09]	-0.02 [-0.11; 0.08]	-0.10 [-0.35; 0.15]
Nr. of cold days × incubation period	→ Dependent variable	0.24 [-0.05; 0.53]	-	-	-	-
<i>Date</i>	→ <i>Dependent variable</i>	<i>-0.04 [-0.21; 0.14]</i>	<i>-0.13 [-0.37; 0.10]</i>	<i>-0.30 [-0.63; 0.03]</i>	<i>0.04 [-0.15; 0.23]</i>	<i>-0.16 [-0.61; 0.29]</i>
<i>Incubation period/ Nestling period</i>	→ <i>Dependent variable</i>	<i>-0.20 [-0.46; 0.06]</i>	<i>-0.15 [-0.38; 0.09]</i>	<i>0.22 [-0.07; 0.51]</i>	<i>0.41 [0.03; 0.79]</i>	-
<i>Brood size</i>	→ <i>Dependent variable</i>	-	-	<i>0.03 [-0.11; 0.17]</i>	<i>0.18 [-0.07; 0.43]</i>	-
Date	→ Daily mean temperature	0.86 [0.27; 1.44]	0.85 [0.31; 1.38]	0.84 [0.74; 0.94]	0.84 [0.30; 1.38]	0.85 [-0.15; 1.85]
Date	→ Total amount of precipitation	0.20 [-0.09; 0.48]	0.05 [-0.11; 0.21]	0.05 [-0.09; 0.18]	0.05 [-0.11; 0.20]	0.01 [-0.25; 0.27]
Daily mean temperature	→ Nr. of hot days	0.64 [0.14; 1.15]	0.74 [0.24; 1.25]	0.75 [0.61; 0.89]	0.75 [0.24; 1.26]	0.82 [-0.17; 1.80]
Daily mean temperature	→ Nr. of cold days	-0.65 [-1.16; -0.14]	-0.43 [-0.82; -0.05]	-0.44 [-0.58; -0.29]	-0.44 [-0.83; -0.05]	-0.47 [-1.22; 0.28]
Total amount of precipitation	→ Nr. of dry days	-0.42 [-0.83; -0.01]	-0.39 [-0.75; -0.02]	-0.39 [-0.63; -0.16]	-0.39 [-0.76; -0.02]	-0.43 [-1.15; 0.28]
Total amount of precipitation	→ Nr. of heavy rain days	0.92 [0.31; 1.52]	0.92 [0.36; 1.48]	0.93 [0.83; 1.03]	0.93 [0.37; 1.49]	0.91 [-0.13; 1.95]
Nr. of cold days	→ Incubation period	0.36 [-0.02; 0.74]	-	-	-	-
Nr. of cold days	→ Interaction	0.96 [0.34; 1,58]	-	-	-	-
Incubation period	→ Interaction	0.09 [-0.10; 0.27]	-	-	-	-

5

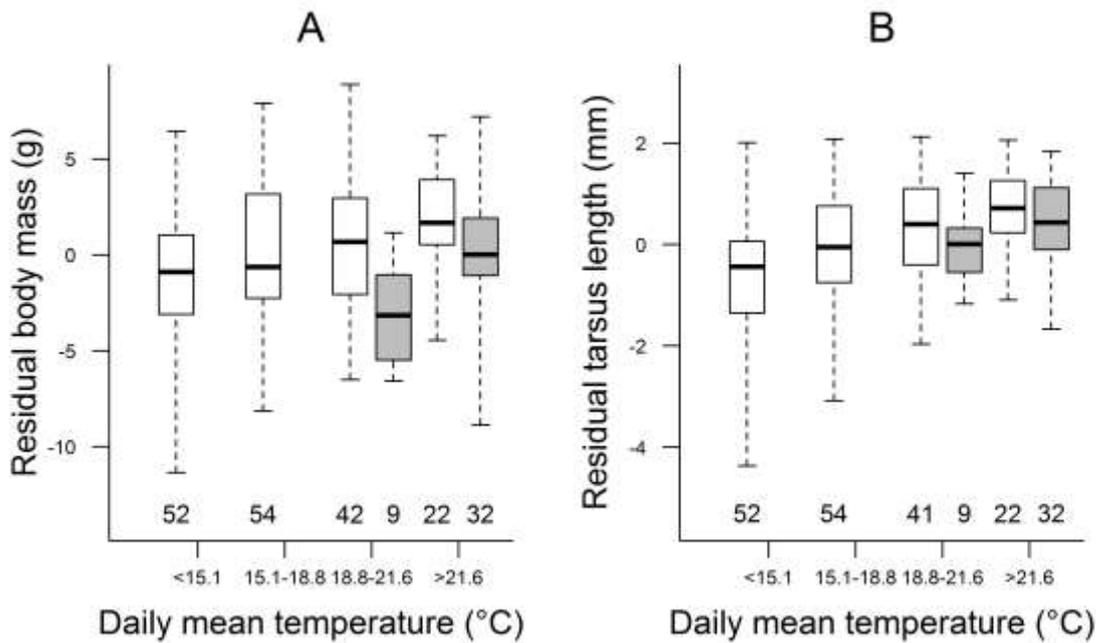


Figure 3.4. Relationship of nestlings' body size with average daily mean temperature and number of hot days. For illustrative purposes, daily mean temperature was categorized according to its 25%, 50% and 75% percentiles. The number of hot days was dichotomized as few (≤ 2 ; white boxes) and many (> 2 ; grey boxes) as the median was zero and the 75% percentile was 2 hot days. Body mass (A) was controlled for date and age of nestlings, whereas tarsus length (B) was controlled for brood size and age of nestlings by taking their residuals from linear regressions. Box plots show the median (thick line), interquartile range (box) and the range of data (whiskers); sample sizes (i.e. number of broods) are shown below each box.

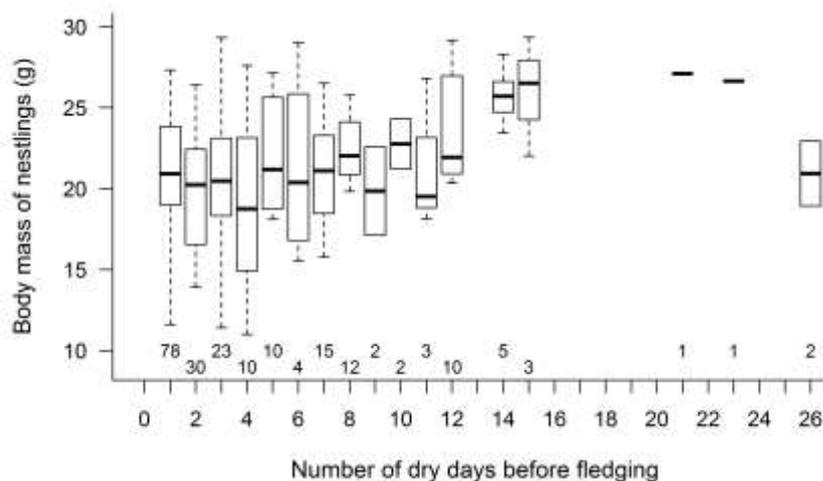


Figure 3.5. Relationship of nestlings' body mass with the number of dry days before fledging. Box plots show the median (thick line), interquartile range (box) and the range of data (whiskers); sample sizes are shown below each box.

Sex ratio of the 285 nestlings with known sex did not differ significantly from unity when all years were combined (binomial test: 140 males, 49.1%, $p = 0.813$), and did not differ between years (χ^2 test: $\chi^2_2 = 1.09$, $p = 0.581$; Table S6). Similarly, the primary sex ratio of 20 clutches (where all the laid eggs hatched) did not differ significantly from unity (40 males out of 85 nestlings, 47%; $p = 0.665$). Brood sex ratio did not show considerable relationship with any of the studied meteorological variables for house sparrows (Table 3.1, Table A/1.6). Multigroup analyses showed that models assuming sex-dependent weather effects on nestlings' body size did not fit the data better than models with sex-independent parameter estimates either for body mass ($\hat{C}_{\min}=6.63$, $p=0.250$) or tarsus length ($\hat{C}_{\min}=0.59$, $p=0.964$), indicating no sex difference in nestling size in response to weather.

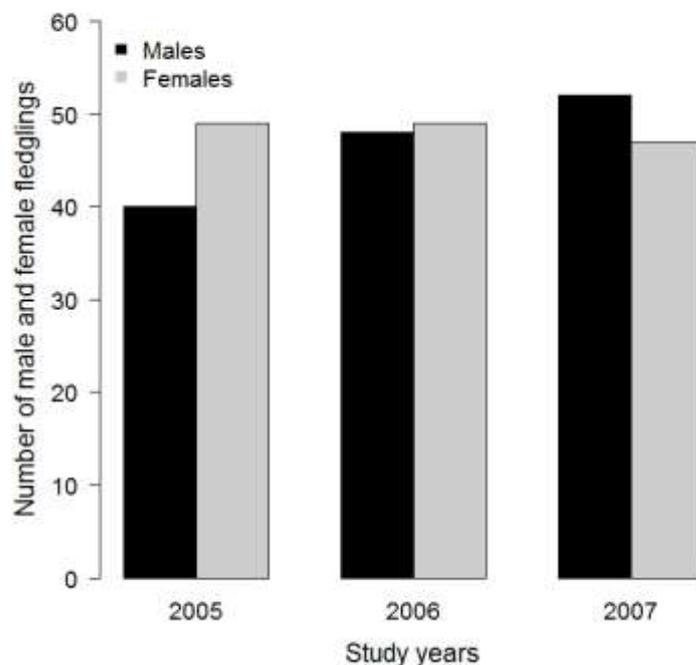


Figure 3.6. Number of male and female house sparrow nestlings in different study years

Discussion

Our study has revealed several correlations between weather conditions and various components of reproductive success of house sparrows. Our results suggest that warm weather was generally favourable both during the incubation and nestling periods, but extreme heat had a negative effect on nestlings' body size, whereas dry periods up to 2-3 weeks resulted in higher nestling weight. The effect sizes of these relationships were small, but in some cases comparable to the effects of other important determinants of reproductive

success such as date and brood size. Furthermore, the 95% confidence intervals of the path coefficients indicated that temperature can have strong effect on nestlings' size. Interestingly, our results indicate that extreme temperatures (i.e. those occurring only in 10% of time in our temperate region) can have differential effects on different aspects of avian reproduction, and these effects can oppose the general effect of average daily temperatures.

Hatching success increased with the number of hot days, probably because $>30^{\circ}\text{C}$ air temperatures help to maintain the optimal temperature of eggs. The average incubation temperature of house sparrows is 34.2°C (Anderson 2006). When parents are not incubating, the temperature of eggs may decrease less if the weather is warm, leading to lower variability in egg temperature and thereby better embryo development. Similarly, hatching success decreased with the number of cold days, but only when the incubation period was relatively short. This interaction probably arose because both hatching success and the length of the incubation period may be related not only to weather but also other variables such as the body condition, age and experience of parents (Martin 1987; Deeming 2008). Thus, clutches with incubation periods prolonged due to cold days may be more successful than those prolonged due to poor parental quality, leading to a spurious positive relationship between the number of cold days and hatching success for long incubation periods.

Fledging success was not related to weather conditions which is surprising as weather extremities can affect nestling mortality directly and also indirectly through food availability in other species (Elkins 2004; McDonald *et al.* 2004). A study of British house sparrows (Peach *et al.* 2008) found a quadratic relationship between the annual number of independent young raised per pair and both temperature and precipitation within a similar range as our meteorological data. However, since the house sparrow is a multi-brooded species, annual reproductive output is affected by the number of broods raised per year which in turn may also be influenced by weather (Zahn *et al.* 1995; Reid *et al.* 2000), a phenomenon we could not study because not all parents were ringed in our population. Over our 6 study years, length of the reproductive season (from the laying the first egg until the ringing of the last nestling in the colony) tended to increase with yearly mean temperature (Pearson correlation: $r=0.77$, $p=0.075$), which suggests at least the possibility of a similar temperature effect in our population as those found in Britain.

The correlations we found indicate that, at least within a single reproductive attempt, weather variability in our region has little effect on the proportion of young that reaches the age of fledging, but it can strongly affect the quality of offspring, as both body mass and tarsus length of nestlings were greater under warm weather conditions (i.e. higher mean daily

temperature). Larger fledglings have better survival (Ringsby, Saether & Solberg 1998; Schwagmeyer & Mock 2008; Mock & Schwagmeyer 2010), so the size and body condition of offspring is an important component of parents' fitness. Warm weather may promote nestling growth by several mechanisms. First, house sparrow nestlings are poikilothermic for the first ca. 10 days of their life and need brooding from parents to maintain their body temperatures in the thermoneutral zone of 35-40°C (Anderson 2006). Higher air temperatures may reduce the heat loss of unattended broods and thereby may allow both nestlings and parents to invest less into thermoregulation and more into growth and foraging. Second, nestling development is dependent on provisioning by the parents. In house sparrows, investment by both parents is required to maximize reproductive success (Hoi *et al.* 2003), and unfavourable weather conditions reduce the provisioning rate of male parents (Pipoly *et al.* 2011). Nestlings of this species require a diet of >80% arthropod prey (Anderson 2006; Brzek *et al.* 2009), and weather may affect the activity and abundance of many arthropod taxa resulting in lower success of finding food for nestlings in cold, wet and windy weather (Holm & Edney 1973; Elkins 2004; Chalfoun & Martin 2007; Winkler, Luo & Rakhimberdiev 2013).

Beside the positive effects of generally warm weather, we found that the frequency of extremely hot days was negatively related to nestlings' body size. This is probably due to heat stress, as increasing temperature triggers higher metabolic rate (Anderson 2006) and extreme heat can cause heat shock (Murphy 1985; Lindquist 1986). It seems unlikely that extreme hot temperature reduces the availability of nestlings' food since insects are generally active in warm weathers (Taylor 1963; McIntyre 2000). However, we need further information on parental behaviour, since if parental activities on extremely hot days (e.g. hunting for chicks' food) have significant physiological costs then parents may reduce provisioning even at the expense of reduced growth or survival of current broods.

Precipitation had negligible effect on all aspects of reproduction in our study except for the body mass of nestlings: the longer the period of uninterrupted dry weather before fledging, the larger the fledglings' weight. A possible explanation for this relationship is that insects may be hidden and immobile in rain, thus dry weather may increase food availability and thereby the body condition of nestlings at ringing.

Although adult sex ratio is usually slightly male-biased in house sparrow populations (Anderson 2006), in our study the sex ratio of neither hatchlings nor fledglings differ significantly from unity. In parallel with our result that nestling mortality (i.e. fledging success) was unrelated to meteorological variables, we found that offspring sex ratio did not vary with weather conditions. In line with this, in a North-American population of house

sparrows, Westneat et al. (Westneat *et al.* 2002) found little evidence that offspring sex ratio is shifted under good conditions, measured by date and parental characteristics. Furthermore, our results indicated that the effects of weather on nestlings' body size was similar in male and female nestlings. Altogether, these findings do not support that the two sexes differ considerably in environmental sensitivity during early ontogeny and/or in parents' investment into offspring in this species.

Although correlative studies cannot prove causation, our study highlights the importance of the deeper understanding of weather effects on avian reproduction. For example, the effects of meteorological conditions may be complicated by variation in spring phenology. Although breeding date *per se* had little effect on reproduction in our analyses except that fledglings' body mass decreased over the season (Table 1), the different timings of birds' egg laying and arthropods' emergence (i.e. a mismatch in phenology) can be an important determinant of fitness (Both *et al.* 2006; Møller *et al.* 2008). Mismatches can potentially confound the effects of weather, e.g. because warm temperatures might promote nestling growth via food availability in well-timed breeders but not (or less so) in mismatched pairs or years. Further studies are needed to tease apart these effects.

Taken together, we found complex relationships between weather and the reproductive success of house sparrows. Our results indicate that the overall meteorological conditions and extremities can have opposing effects which can vary between different components of fitness. This implies that the consequences of globally rising temperatures and increasing frequency of extreme meteorological conditions are not easy to predict, and detailed studies at the population level are necessary for a better understanding of the impact of weather and climate on population dynamics.

4. Effects of extreme heat on the reproductive output of great tits (*Parus major*, *L.*) breeding in urban and natural habitats

Introduction

Global climate change is expected to result in an increased incidence of extreme weather events, such as heat waves, cold spells and droughts (see section 1.1). When these events occur during the late spring and early summer, they can have detrimental effects on reproductive success in species for which this period is the peak breeding season, like many birds. A single event (usually) might not cause population-level decline, but can influence the short-term reproductive output of individuals e.g. due to loss of some of their actual offspring. If several extreme events occur or an extreme event takes long time in a single breeding season, majority of breeding individuals may die or may not successfully raise offspring which then can have population-level consequences (Møller 2011; Gładalski *et al.* 2018).

Heat-related mortality of animals can be significant (McKechnie, Hockey & Wolf 2012). Birds, because of their usually small body size and predominantly diurnal habits, are particularly vulnerable to extreme heat waves. For example, a “famous” heat wave occurred in southern and western Europe in 2003, when a prolonged and exceptionally intense heat wave resulted in 70,000 heat-related deaths of humans (Robine *et al.* 2008), and decreased populations of several bird species as well (Jiguet *et al.* 2006). In Australian passerine birds, adult survival was associated with temperature extremes more strongly than with average temperatures (Gardner *et al.* 2017), and in North American grassland birds nest survival rate was negatively associated with hot and dry weather both within and across breeding seasons in North American grassland birds (Conrey *et al.* 2016). A study found that offspring recruitment to the breeding population of long-tailed tits (*Aegithalos caudatus*) in the United Kingdom was associated with breeding season temperature (Gullett *et al.* 2015), so it seems that weather conditions during pre-fledging period can have long-term effects on offspring survival.

Compared to survival, a much less investigated phenomenon is the effect of extreme weather on the quality of reared offspring. Long-term fitness of individuals may be affected even if extreme weather events do not cause mortality but reduce the quality of offspring. As the size and body mass of nestlings are correlated with post-fledging survival (Perrins 1965; Tinbergen & Boerlijst 1990; Linden, Gustafsson & Part 1992), weather conditions during nestling development can influence fitness via affecting nestling size and/or body mass. A study found that morphometric traits of juvenile cliff swallows (*Petrochelidon pyrrhonota*)

were not highly sensitive to weather conditions during a five-year investigation period, although nestlings were smaller in cooler years (Roche, Brown & Brown 2014). An other study on great tits showed that the body mass of offspring was lower in heated than control nests (Rodríguez & Barba 2016), but see also chapter 3.

Urban and natural areas are characterized by fundamental differences in their ecological features (see section 1.1). Urban areas are warmer than surrounding natural areas due to the urban heat island (UHI) effect, and there is some evidence in humans that heat waves have greater impact on mortality rates in highly built-up big cities than in rural settlements (Gabriel & Endlicher 2011). The UHI effect is especially important during the summer months when breeding of many birds occurs, and is usually more evident at night. The intensity of a city's heat island effect is dependent on the size of the city and its building density. During heat waves the local effect of an UHI is superimposed on the regional temperature, thereby the urban environment exacerbates extreme heat events. Thus, urban animals are more likely to reach their upper limit of thermal tolerance (Krause *et al.* 2017). However, to our knowledge, research comparing the effects of temperatures on bird populations living in urban and non-urban habitat types is completely lacking.

Constant exposure (i.e. over successive days) to high temperatures can lead to reductions in adult body mass, as well as affect nestling development. These phenomena can emerge for example through a combination of dehydration via increased rates of evaporative cooling, energetic constraints that result from reduced foraging opportunities or provisioning effort (Kruuk, Osmond & Cockburn 2015; Gardner *et al.* 2017), or through the cost of higher metabolic rate on higher temperatures (Merckx *et al.* 2018). Because urban environments are on average warmer, and the main food of insectivorous birds' offspring, e.g. caterpillars, may have much lower abundance in cities than in deciduous woodlands (Seress *et al.* 2018), extreme hot weather can be expected to exert stronger negative effects on the quality and survival of nestlings in urban areas than in more natural habitats.

In this study, our aims were 1) to investigate the effects of extreme high temperatures on breeding success of great tits measured as nestlings' size and survival, and 2) to assess if these effects differ between cities and non-urban forest habitats. We analysed breeding biology data of great tits from two urban and two forest populations from 2013 to 2018, and we took into account other, non-weather related factors that differ between the two habitat types. As urban areas are warmer due to UHI, and reproductive performance of urban animals is often lower in cities compared to more natural areas (Seress *et al.* 2012; Bailly *et al.* 2016), we predicted greater negative impact of extreme heat on reproduction in urban than natural habitats.

Methods

Meteorological data

To analyse the effects of weather conditions on breeding success, we calculated weather variables for the nestling period (from the day of hatching of first chick to the day preceding the day of the last chick's death or the day of ringing and measuring the chicks) for each brood. We used the hourly temperature (°C) records of our weather stations (see in section 2.1) at each study site to calculate the average temperature for each brood as the mean of our hourly temperature data over the nestling period. To quantify extreme heat, we defined a reference period from the earliest hatching date to the latest chick ringing date in the total of our six-year data set, so the reference period was from 9th April to 15th July. To estimate the typical temperatures in our geographic area, we used a 26-years dataset from an external reference weather station located in Szentkirályszabadja, a small village near Veszprém (47°57'06" É, 17°58'10" K, ca. 9.5 – 22 km from our study sites). The weather station was maintained by the Hungarian Meteorological Service and temperature data of this weather station was available from the NOAA database (www.ncdc.noaa.gov), recorded every three hours a day (0, 3, 6, 12, 15, 18, 21 UTC) in 1993-2018. We calculated the 90 % percentile of daily maximum temperatures for the reference period from this 26-year long dataset, and this percentile value (28.7 °C) was used as a threshold for defining extreme hot days during chick rearing. Thus, we calculated the number of hot days in each nestling period as the number of days when the daily maximum temperature was higher than 28.7 °C.

Measuring reproductive success

Data collection in the field is described in section 2.1. For analysing the effects of weather on fledging success, we used broods where at least one offspring hatched and at least one offspring was alive on the third day after the hatching of the first nestling in that brood. We omitted the broods that failed within the first three days after hatching because the average interval between our nest monitoring visits was three days, so estimating the number of hatched chicks and their survival before the first nest check after hatching would be very uncertain. We could include n=760 broods for fledging success analyses, from which 385 had no chick mortality, 319 had partial mortality and 56 had complete brood loss during nestling rearing.

For analysing the effects of weather on the size of the nestlings, we used the average body mass and the average tarsus length of each brood as response variables, and we included broods where at least one offspring was alive at the age of 14-16 days (when the nestlings were ringed and measured). Throughout the study, “reproductive success” will be used as a

collective term for both the survival and the size of the juveniles, however, the size of the nestlings is influenced by the genetic value of their parents as well. Because chick size varies with age, we omitted those few broods where the nestlings were measured before or after 14-16 days of nestling age ($n=28$). We had $n=674$ broods for nestling size analyses.

Statistical analyses

Investigating the effects of extreme weather is challenging. Extreme events are rare by definition, so their distribution is strongly skewed. Furthermore, weather variables can be correlated with each other and with other seasonal variables that can influence reproductive success, e.g. hatching date, so multicollinearity can be another problem in models containing multiple predictors. Statistical methods that can handle multicollinearity, such as covariance-based structural equation modelling (SEM), are less well suited for handling non-normal data distributions and the non-independence structure of ecological data (e.g. multiple broods per pair). Therefore, as a first approach to this problem, we used generalized linear mixed-effects (LME) models, which can be applied appropriately for non-normal and non-independent data, although less suitable for separating the effects of correlated predictor variables than the SEM approach.

For average nestling mass and average tarsus length of nestlings as response variables, we used LME models with Gaussian error. For fledging success (proportion of hatched chicks that were alive at the age of 14-16 days) as response variable, we used LME models with quasibinomial error distribution and logit link function. All of our models contained pair identity as random factor to control for the non-independence of broods that had the same parents (there were 111 pairs of parents that had more than one brood in our dataset, ranging 2-6 broods/ pair). Our main predictors of interest were the number of hot days (used as a numeric covariate) and study site (two cities and two forests), and their two-way interaction. We built two types of models for each response variable: 1) a simple model with no multicollinearity that contained only our main predictors of interest, i.e. the number of hot days and study site and their two-way interaction, and 2) a complex model that contained further potentially important predictor variables. In the latter model, we included year as categorical variable (6 years), and hatching date as a numeric covariate defined as the number of days to the first hatched nestling in each brood from 1st January annually. For models of nestling size (i.e. average body mass and average tarsus length of nestlings) we also included two further numeric covariates: brood size as the number of offspring at fledging (i.e. number of measured nestlings), and brood age as the number of days from hatching of the first chick to measuring the fledglings (ranging 14 to 16 days). In the model of fledging success, we added

the quadratic term of hatching date, because preliminary graphical inspection of the data suggested a non-linear seasonal change in fledging success. Variance inflation factor (VIF), a measure of multi-collinearity, ranged from 1.04 to 2.85 in these complex models. We did not incorporate the average temperature during each nestling period in the models, because average temperature correlated with both the number of hot days and date, leading to high multi-collinearity in some of the models (VIF: 1.04 – 6.24).

To statistically compare the effect of hot days between the two habitat types, we calculated a linear contrast from each model's estimates (i.e. the difference between the two urban sites versus the two forest sites). We used this approach rather than including habitat type as a fixed effect and site as a random effect because variance estimations of random effects with few levels are unreliable (Piepho, Büchse & Emrich 2003; Bolker *et al.* 2008), whereas including both habitat type and site as fixed effect would have resulted in a model with high collinearity between these two factors (Dormann *et al.* 2012). Instead, we treated statistically the four sites similarly as if they were two control groups and two treatment groups in an experiment, and we used a pre-planned comparison to test the prediction that the two treatment (i.e. urban) groups would differ from the two control (i.e. forest) groups. Note that pre-planned comparisons are a powerful approach for testing a priori hypotheses (Ruxton & Beauchamp 2008). All analyses were implemented in the R 3.1.1 software environment (R Core Team 2014), using packages “nlme” (Pinheiro *et al.* 2013), “MASS” (Ripley *et al.* 2013) and “emmeans” (Lenth 2018).

Results

Urban and forest habitats had markedly different temperature profiles with an average 1.53 °C higher temperature in urban than in forest habitat over the whole 6 year study period. Average temperatures of the nestling periods were significantly different between habitat types and between study years, too (Figure 4.1). The number of hot days was also significantly different between habitat types and among years as well (Figure 4.2), with more frequent hot days in urban than in forest habitat. Out of the 390 urban nestling periods, at least one hot day occurred in 179 periods (45.9%), whereas out of the 370 forest nestling periods, at least one hot day occurred in 77 periods (20.8 %). Number of hot days ranged between 0 and 13 in urban nestling periods, and between 0 and 5 in forest nestling periods (Figure 4.2), and the highest maximum temperature was 40.1 °C in urban (in June 2013) and 33.6 °C in forest nestling periods (in May 2014).

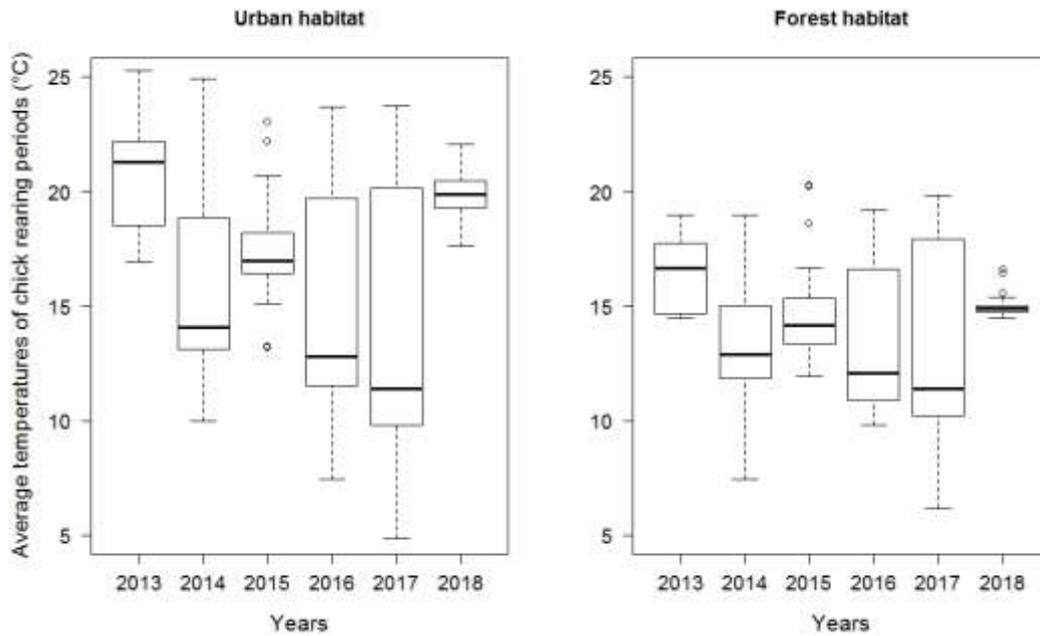


Figure 4.1. Distribution of the average temperature of nestling periods in each year in urban and forest habitats. Temperature significantly differed between years within habitat (ANOVA, urban: $F_{5,389} = 24.693$, $P < 0.001$; forest: $F_{5,369} = 11.772$, $P < 0.001$), as well as between habitats (ANOVA: $F_{1,759} = 41.993$, $P < 0.001$), with an average 1.53 °C higher temperatures in urban than in forest habitats.

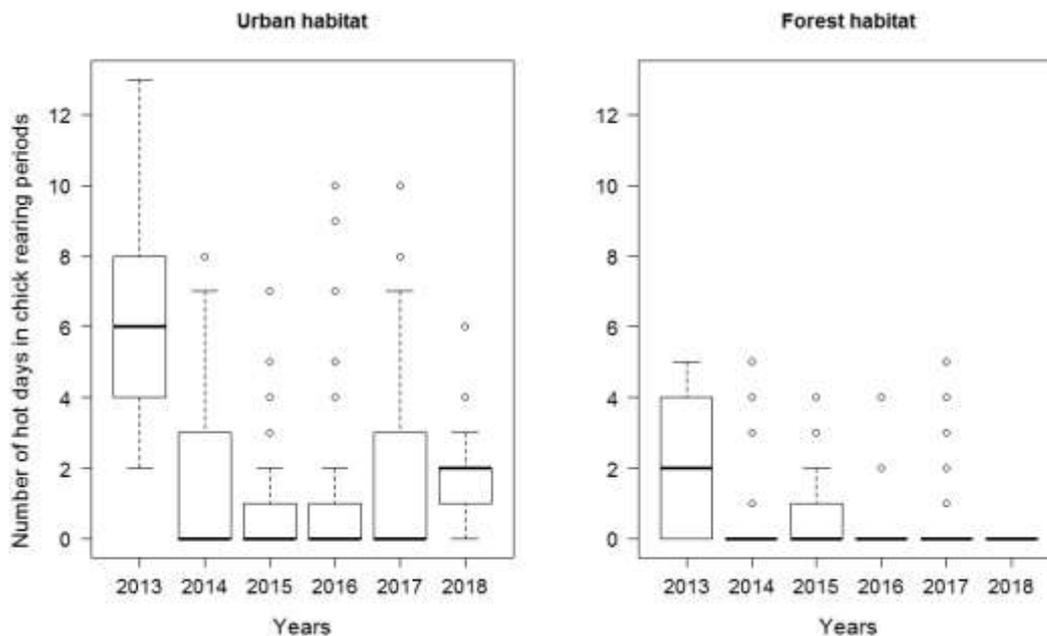


Figure 4.2. Distribution of the number of hot days during nestling periods in each year in urban and forest habitats. Number of hot days significantly differed between years within habitat (ANOVA, urban: $F_{5,389} = 25.915$, $P < 0.001$; forest: $F_{5,369} = 11.676$, $P < 0.001$), as well as between habitats (ANOVA: $F_{1,759} = 77.959$, $P < 0.001$), with an average 1.12 day difference between urban and forest habitats.

Our data suggest that the effect of extreme hot periods on reproductive success varies among sites. The number of hot days always showed positive relationship with the measured components of reproductive success in the urban population of Balatonfüred (the hottest site). In this study site, chick mass increased significantly with increasing number of hot days, and the other two reproductive components had non-significant positive relationships with the number of hot days. The reproductive success of the other three sites showed mostly negative relationships with number of hot days, although many of these relationships were not statistically significant (Table 4.1 & 4.2, Figure 4.1). The magnitude of negative effect of hot days varied among reproductive success components, and also between simple and complex models (Table 4.1 & 4.2). Chick mass decreased with increasing number of hot days according to both the simple and the complex models (Table 4.1 & 4.2, Figure 4.3), and this relationship was significant in all sites according to the complex models. The average tarsus length of chicks in a brood was only affected by number of hot days in the urban site Veszprém where it significantly decreased with increasing number of hot days according to the simple model (Table 4.1 & 4.2, Figure 4.3). Fledging success was significantly lower with increasing number of hot days at the urban site Veszprém and at the forest site Vilma-puszta according to simple models, although these relationships became non-significant in the complex models (Table 4.1 & 4.2, Figure 4.3).

Comparing the overall (average) effects of extreme heat between urban and forest populations with linear contrasts suggest significant habitat differences (Table 4.3). According to the simple models, the negative effect of increasing number of hot days on average chick mass was significantly stronger (Table 4.3) in forest compared to urban populations (which is consistent with the more negative slopes obtained for the two forest sites; Table 4.1). For the average chick tarsus length, we did not find significant habitat difference in the effect of hot days (Table 4.3). The decrease in fledging success with increasing number of hot days was significantly stronger in forest compared to urban habitat in the simple model (Table 4.3). Note that this latter difference is driven by Balatonfüred, since the negative slope for the other urban site (Veszprém) falls between the estimates for the forest sites (Table 4.1). In the complex model, the difference in effect of hot days on fledging success between urban and forest habitat was not significant, although it had similar direction as in the simple model (Table 4.3). All results remained qualitatively the same if we re-ran the analyses without the potential outlier in Balatonfüred (Figure 4.3). The effects of other predictor variables on chick size and fledging success are given in Table 4.4.

Table 4.1. Relationship between measures of reproductive success and the number of hot days during the nestling period, as estimated by marginal means from the *simple models* (including only the interaction between number of hot days and study site).

Estimates with standard error (SE) refer to the slope of response variable with the number of hot days in each study site (the same slopes are shown in Fig. 4.3). Slopes significantly different from zero (i.e. zero not included between the lower and upper limit of the 95% confidence interval (CI) of the slope estimate) are highlighted in bold. Linear contrasts (Table 4.3) for habitat comparison were calculated from these parameter estimates.

Study sites	Chick mass				Chick tarsus length				Fledging success			
	Estimate	SE	lower CI	upper CI	Estimate	SE	lower CI	upper CI	Estimate *	SE	lower CI	upper CI
Veszprém city	-0.081	0.046	-0.172	0.010	-0.052	0.021	-0.094	-0.011	-0.107	0.049	-0.204	-0.009
Balatonfüred city	0.163	0.049	0.065	0.260	0.036	0.023	-0.009	0.081	0.002	0.055	-0.106	0.110
Szentgál forest	-0.106	0.085	-0.273	0.061	-0.050	0.038	-0.126	0.026	-0.029	0.130	-0.286	0.228
Vilma-pusztá forest	-0.489	0.131	-0.749	-0.230	-0.059	0.060	-0.178	0.060	-0.507	0.150	-0.802	-0.212

For chick mass and chick tarsus length, number of pairs was 535 and number of broods was 674, df = 135. For fledging success, number of pairs was 600 and number of broods was 760, df = 156

* Estimates of fledging success are on the logit scale

Table 4.2. Relationship between measures of reproductive success and the number of hot days during the nestling period, as estimated by marginal means from the *complex models*.

Estimates with standard error (SE) refer to the slope of response variable with the number of hot days in each study site. Slopes significantly different from zero (i.e. zero not included between the lower and upper limit of the 95% confidence interval (CI) of the slope estimate) are highlighted in bold. Linear contrasts (Table 4.3) for habitat comparison were calculated from these parameter estimates.

Study sites	Chick mass				Chick tarsus length				Fledging success			
	Estimate	SE	lower CI	upper CI	Estimate	SE	lower CI	upper CI	Estimate *	SE	lower CI	upper CI
Veszprém city	-0.123	0.057	-0.236	-0.009	-0.050	0.026	-0.102	0.001	0.079	0.069	-0.057	0.215
Balatonfüred city	0.116	0.053	0.012	0.221	0.029	0.024	-0.018	0.076	0.089	0.067	-0.044	0.221
Szentgál forest	-0.256	0.096	-0.446	-0.066	-0.070	0.043	-0.156	0.015	0.089	0.157	-0.222	0.400
Vilma-pusztá forest	-0.401	0.130	-0.659	-0.143	0.011	0.059	-0.105	0.128	-0.225	0.153	-0.528	0.079

For chick mass and chick tarsus length, number of pairs was 535 and number of broods was 674, df = 127. For fledging success, number of pairs was 600 and number of broods was 760, df = 149

* Estimates of fledging success are on the logit scale

Table 4.3. Linear contrasts comparing the effect of the number of hot days between urban and forest habitats. Each contrast (d, with its standard error, SE) expresses the difference between the two urban and two forest populations in the slope of relationship between reproductive parameters and the number of hot days. Positive contrasts mean more positive slopes in the urban habitat or more negative slopes in the forest habitat.

Response	Simple model			Complex model		
	d ± SE	t	p	d ± SE	t	p
chick body mass	0.338 ± 0.085	3.978	< 0.001	0.325 ± 0.082	3.978	< 0.001
chick tarsus length	0.046 ± 0.039	1.187	0.237	0.018 ± 0.037	0.503	0.616
fledging success	0.215 ± 0.106	2.037	0.043	0.152 ± 0.108	1.401	0.163

Simple models: For chick mass and chick tarsus length, df = 135, for fledging success, df = 156
 Complex models: For chick mass and chick tarsus length, df = 127, for fledging success, df = 149

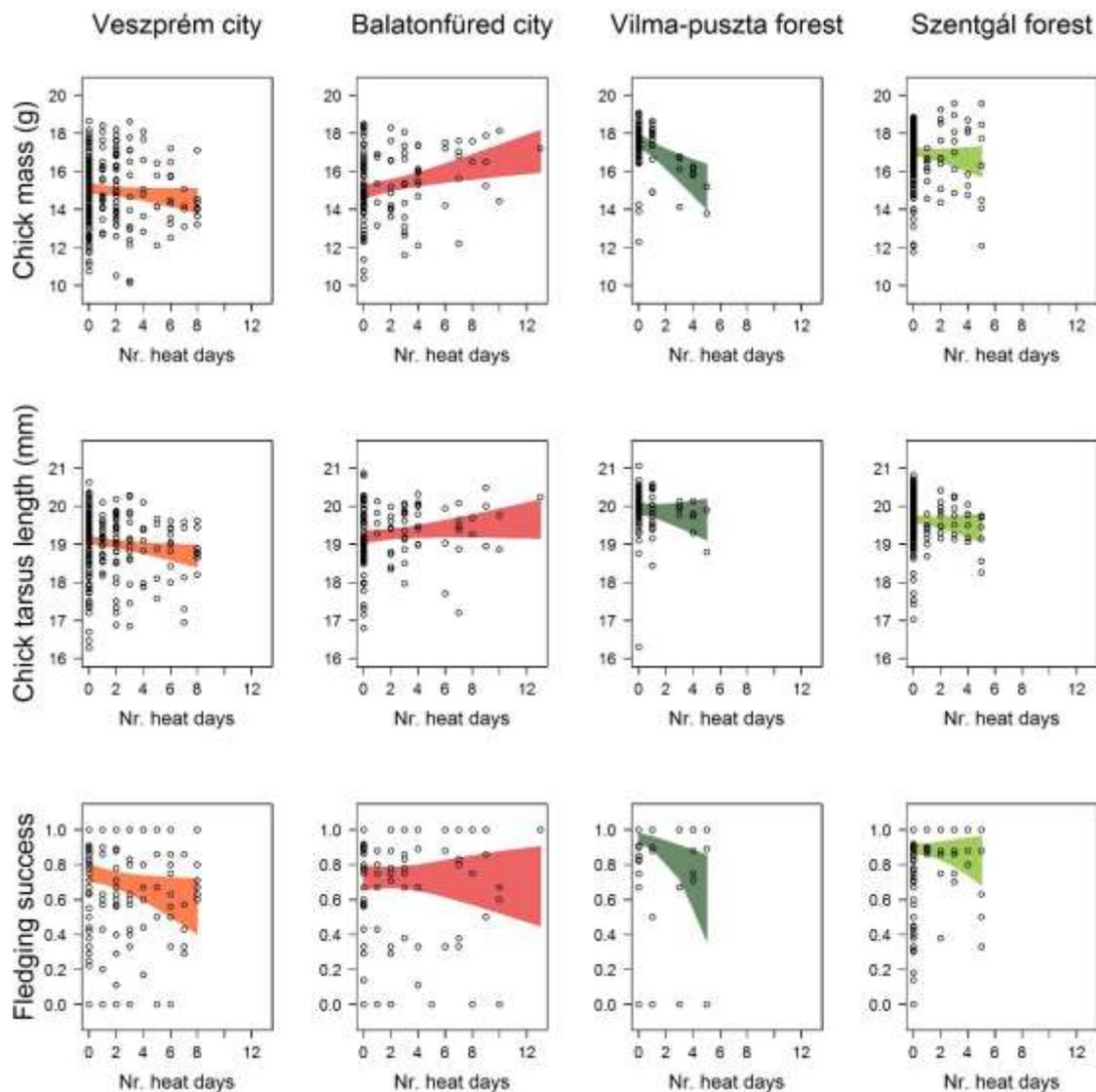


Figure 4.3. Relationship of average body mass (upper panels), average tarsus length (middle panels) and fledging success (bottom panels) of broods with number of hot days in each study site. Coloured stripes show the 95% confidence band of the slope of relationship estimated from the simple models (Table 4.1).

Table 4.4. Parameter estimates of the complex models of great tit reproductive success variables in relation to the number of hot days and other predictors. The intercept refers to the urban site Veszprém and year 2013 when all numeric predictors are zero; all other parameters refer to the difference from the intercept. β is the parameter estimate, SE is the standard error, DF is the degrees of freedom. Sample size is $n=674$ broods with $n=535$ pairs for nestling size and $n=760$ broods with $n=600$ pairs for fledging success. Estimates of fledging success are on logit scale.

	Chick mass					Chick tarsus length					Fledging success				
	β	SE	DF	t	p	β	SE	DF	t	p	β	SE	DF	t	p
(Intercept)	10.413	1.465	531	7.110	0.000	16.548	0.656	531	25.211	0.000	-10.094	3.988	596	-2.531	0.012
brood size	0.077	0.029	127	2.710	0.008	0.082	0.013	127	6.440	0.000	-	-	-	-	-
brood age	0.334	0.082	127	4.095	0.000	0.142	0.037	127	3.871	0.000	-	-	-	-	-
hatching date	0.004	0.005	127	0.777	0.439	0.003	0.002	127	1.263	0.209	0.207	0.061	149	3.370	0.001
hatching date ²	-	-	-	-	-	-	-	-	-	-	-0.001	0.000	149	-3.784	0.000
year 2014	-0.494	0.295	127	-1.673	0.097	-0.318	0.133	127	-2.396	0.018	-0.556	0.473	149	-1.175	0.242
year 2015	-0.915	0.311	127	-2.941	0.004	-0.219	0.140	127	-1.566	0.120	-0.800	0.506	149	-1.579	0.116
year 2016	-1.067	0.302	127	-3.530	0.001	-0.217	0.135	127	-1.605	0.111	-1.148	0.476	149	-2.410	0.017
year 2017	-1.479	0.323	127	-4.587	0.000	-0.448	0.144	127	-3.105	0.002	-2.226	0.466	149	-4.774	0.000
year 2018	-0.773	0.323	127	-2.394	0.018	-0.275	0.144	127	-1.910	0.058	-1.232	0.490	149	-2.512	0.013
nr. hot days	-0.123	0.057	127	-2.144	0.034	-0.050	0.026	127	-1.948	0.054	0.079	0.069	149	1.149	0.253
site Balatonfüred	-0.144	0.211	531	-0.682	0.496	0.082	0.093	531	0.878	0.380	-0.114	0.235	596	-0.487	0.626
site Szentgál forest	1.790	0.188	531	9.530	0.000	0.390	0.083	531	4.696	0.000	1.161	0.218	596	5.334	0.000
site Vilma-puszta forest	2.231	0.237	531	9.417	0.000	0.549	0.105	531	5.234	0.000	1.826	0.375	596	4.865	0.000
nr. hot days : site Balatonfüred	0.239	0.065	127	3.670	0.000	0.079	0.029	127	2.686	0.008	0.010	0.080	149	0.123	0.902
nr. hot days : site Szentgál	-0.133	0.094	127	-1.412	0.160	-0.020	0.042	127	-0.467	0.642	0.010	0.151	149	0.066	0.948
nr. hot days : site Vilma-puszta	-0.278	0.134	127	-2.073	0.040	0.062	0.061	127	1.017	0.311	-0.304	0.160	149	-1.900	0.059

Discussion

We found differences in the effects of extreme hot temperatures on different components of reproductive output, and also between urban and forest populations. Average body mass of broods was smaller when more extreme hot days occurred (except in the urban site of Balatonfüred), but average tarsus length was not affected by number of hot days. Fledging success decreased with number of hot days in one urban and one forest populations.

Heat might affect the reproductive output of birds at least two ways: indirectly through food amount and availability and directly through nestling physiology. According to studies about reactions to extreme high temperatures in lepidopteran larvae (which are the main source for nestling diet in great tits and many other birds), one of the most consistent result is that increasing temperature strongly decreases the time to pupation in several lepidopteran species (Kingsolver *et al.* 1997; Lee & Roh 2010; Lemoine, Capdevielle & Parker 2015), so optimal caterpillar food may be available for shorter time to insectivorous passerines when the temperature is hot due to frequent hot days. Additionally, growth and food consumption of caterpillars can decline rapidly above a critical temperature (Kingsolver *et al.* 1997) as well as their mortality can increase when temperature is constantly high (York & Oberhauser 2002; Lee & Roh 2010). So, it is possible that avian nestlings have lower body mass when there are extreme hot conditions due to shorter time when prey is available, and this effect can be stronger in forest where the amount of available caterpillar prey is much higher (Seress *et al.* 2018) and can have a stronger decrease because of high temperatures than in urban areas, where nestlings usually get greater proportion of other food types (Sinkovics *et al.* unpublished results). But, if it is true, one can expect reduced nestling growth and/or greater nestling mortality during such a food shortage under hot conditions, but in our study this was not the case as tarsus length of nestlings and fledging success were not affected robustly by number of hot days. In sum, we suggest that reduced caterpillar biomass due to hot weather may not be the major factor explaining our findings.

It seems more likely that the negative effect of hot days on nestling body mass emerged from the direct physiological effects of heat. Birds are homeothermic, meaning that individuals maintain stable internal body temperatures regardless of external influences. However, the nestlings cannot maintain stable body temperature in the first few days of their life, and their metabolic processes can alter from adults because they're growing organisms (Mertens 1977). So, offspring may more vulnerable to extreme heat than adults, but specific results about the heat response of young nestlings are scarce. Several studies found that even the body temperature of adult birds increased with increasing ambient temperature (Whitfield

et al. 2015; Nilsson, Molokwu & Olsson 2016; Nilsson & Nord 2018), sometimes even exceeding 45°C, which is close to suggested lethal levels for birds. In hot environments, individuals could less effectively dissipate the excess heat as a consequence of increased metabolism, leading to hyperthermia. Increasing metabolism causes decreased utilization of food and faster mobilization of energy reserves, thus can cause lower body mass maybe without influencing other growth parameters. Additionally, during heat stress, the evaporative water loss is elevated as the organism tries to cool itself by evaporation to maintain body temperature in the physiologically normal range, causing dehydration (Weathers 1972; Arad *et al.* 1989). So, increased metabolic rate and water loss both can lead to decreased body mass. In broiler chickens (*Gallus domesticus*), weight gain and food intake were reduced under heat stress (Han & Baker 1993; Quinteiro-Filho *et al.* 2010), suggesting that the cost of heat stress can manifest in reduced body mass, as our study found. In a long-term study with increasing temperature and frequency of extreme heat on a cooperative breeder bird, the superb fairy-wren (*Malurus cyaneus*), heatwave events were associated with reduced chick mass (Kruuk *et al.* 2015). An experiment on great tits found that higher temperatures can impair nestling development, as offspring from heated nestboxes were lighter compared to controls, but the tarsus length of heat treated and control nestlings didn't differ significantly. Additionally, the manipulation of nest microclimate didn't influence parental provisioning behaviour, suggesting that body weight loss was independent from food intake (Rodríguez & Barba 2016). These results are similar to ours and suggest that heat stress has great costs for developing offspring. Additionally, extreme heat may have an effect on the body mass of nestlings indirectly through the physiological response of their parents, as provisioning rate in marsh tits (*Poecile palustris*) seems to potentially be limited by the rate of heat dissipation (Nilsson & Nord 2018).

We also found that the effect of hot days on fledglings' body mass differed between urban and forest populations. Although UHI caused 1.53 °C higher temperature on average and there were more number of hot days in urban compared to forest habitat (see Figure 4.1 & 4.2), hot days affected the reproductive components more negatively in forest populations than in cities. So, contrary to our predictions, non-urban populations seem more vulnerable to extreme heat than urban populations. Urban birds living in constantly warmer environment may have higher heat tolerance due to adaptive thermal evolution and/or phenotypic plasticity, similarly to some invertebrates. For example, in European diving beetles, positive relationship between upper thermal tolerance and adaptability was found (Calosi, Bilton & Spicer 2008). In the water flea (*Daphnia magna*), researchers found higher heat tolerance in

animals living in urban compared to rural habitats and in animals reared at higher temperatures (Geerts *et al.* 2015; Brans *et al.* 2017), while urban acorn ants (*Temnothorax curvispinosus*) exhibited greater heat tolerance and a loss of cold tolerance compared with rural ants (Diamond *et al.* 2017). So, it is possible that urban birds have higher heat tolerance than conspecifics in natural habitats, but this should be further tested in wild bird populations. In our study, the positive effect of hot days in the hottest urban population, and the more negative effects of hot days in forest habitat suggest that urban birds might be adapted to UHI and high temperatures in cities, and thus tolerate the extreme heat better while individuals in natural habitat may suffer greater negative consequences of extreme heat when it occurs.

One other mechanism for heat adaptation in urban habitats may be reduced body size. Urban birds are usually smaller (Liker *et al.* 2008; Chamberlain *et al.* 2009; Bailly *et al.* 2016), and UHI may play a major role in forming this size difference between urban and rural individuals. The increased temperatures associated with UHI result in increased metabolic costs (Birnie-Gauvin *et al.* 2016) and are expected to drive shifts to smaller body sizes (Merckx *et al.* 2018). A study comparing the heat stress reaction of different-sized bird species found that small birds have a more advantageous water economy when environment is hot (Weathers 1972), but see Whitfield *et al.* (2015) where larger species tolerated higher ambient temperatures. In line with the principle of Bergmann's rule, animals with smaller size have higher surface-biomass ratio which facilitates heat loss, thus it can be beneficial against dehydration and overheating in a warmer urban habitat. This idea is supported by a study on several water flea populations which found that smaller individuals were more heat tolerant, and urban individuals were generally smaller than rural ones (Brans *et al.* 2017). In birds, a study on a population of white-browed scrubwrens (*Sericornis frontalis*) also found that smaller individuals survived better when more extreme hot and dry events occurred (Gardner *et al.* 2017). So, it is possible that in our study populations the lack/reduction of negative impact of extreme heat in urban populations emerged because of the smaller size of urban individuals. Further research is needed about long-term consequences of smaller size on recruitment, post-fledging survival and future reproductive success of nestlings that experienced different weather. Also, it remains to be tested if body size is genetically adapted to UHI in birds.

Our study experienced some difficulties of investigating weather effects in urban and natural ecosystems. Future studies about extreme weather effects on wild populations may want to take into account the following issues. First, weather variables can be significantly associated with other variables that affected breeding success, so it would be important to

explore other relevant factors that interact with temperature and/or reproductive success, and these relationships should be taken into account when examining weather effects. Second, several factors other than temperature might have an influence on the lower reproductive output of urban populations, e.g. pollution, fragmentation and predation (Rodewald & Gehrt 2014; Seress & Liker 2015), so correlated characteristics and complexity of these systems should be taken into account when we want to compare weather effects in urban and natural habitats. Finally, extreme heat can have both short- and long-term consequences on populations' reproductive characteristics. There is a great importance of investigating physiological responses of organisms to extreme events and potential mechanisms that can help urban individuals to neutralize the negative effect of high temperatures, in order to predict the scope and degree by which populations will be able to resist future climatic stressors.

5. Effects of habitat urbanisation on the offspring sex ratio of great tits (*Parus major*, L.)²

Introduction

In birds, brood sex ratio is often differ from parity, and the direction and extent of this difference seems to be not random. Females in many birds species appear to optimize the brood sex ratio according to the cost and fitness outcome of producing male and female offspring, which may vary among environments as well as with the quality of the parents (Szász *et al.* 2012). For example, one sex may have higher growth rate than the other, resulting in sexual size dimorphism (one sex having larger body size than the other). This can be one of the main causes of the unequal costs of male and female offspring to parents (e.g. Martins, 2004; Rosivall *et al.* 2004; Råberg *et al.* 2005), as a faster-growing or larger offspring needs larger amounts of food, requiring higher parental effort (e.g. Kalmbach *et al.* 2001). Sexual size dimorphism is widespread in birds, both in eggs (e.g. Cordero *et al.* 2000, 2001) and in nestlings (e.g. larger females: Anderson *et al.* 1997; Massemin *et al.* 2000; larger males: Howe, 1977; Hochachka & Smith, 1991; Badyaev *et al.* 2001; Tschirren *et al.* 2003). Sex differences in offspring survival rate also affect their relative values. For example, different sensitivity of the sexes to environmental stressors like parasites may induce higher nestling mortality in one sex compared to the other. The larger sex is more likely to be the more sensitive one, because there may be a trade-off between growth and immunocompetence, and the larger sex may allocate more resources in the former at the expense of the latter (e.g. Tschirren *et al.* 2003 but see Bize *et al.* 2005). Furthermore, after fledging, the sexes can greatly differ in their dispersal distance (see examples in Végvári *et al.* 2018), mortality and lifespan (e.g. Liker & Székely 2005; Barrett & Richardson, 2011). These components of male and female life history can be highly dependent on environmental factors (for theoretical model see Julliard, 2000). Accordingly, the optimal brood sex ratio can differ between different environments. For example, mothers may produce more offspring of the less vulnerable sex in years or habitats with poor dietary conditions, to optimize their parental investment and increase the number of surviving offspring (Korpimäki *et al.* 2000; Pryke & Rollins 2012). For instance, Komdeur (1996) found in the Seychelles warbler (*Acrocephalus sechellensis*) that producing more females (which remain longer in their natal territories than

² This chapter is based on the manuscript Ágh, N., Pipoly, I., Szabó, K., Vincze, E., Bókony, V., Seress, G., Liker, A. Does offspring sex ratio differ between urban and forest populations of great tits (*Parus major*)?, *Biologia Futura in press* (2020)

males) on low-quality territories reduces the parents' future breeding success, whereas on high-quality territories female offspring stay as helpers, increasing their parents' breeding success. Therefore, parents with high-quality territories are more likely to produce daughters whereas on low-quality territories they produce more sons.

Urban and non-urban habitats often differ in quality and structure, leading to cardinal changes in life history and breeding phenology of birds in anthropogenic environments (Hinsley *et al.* 2008; Chamberlain *et al.* 2009). For instance, urban birds start breeding earlier and have smaller clutches than those in natural habitats (reviewed in Sepp *et al.* 2018, examples for great tit: Bailly *et al.* 2015; Charmantier *et al.* 2017; Seress *et al.* 2018). In cities, body condition of fledglings is often lower and their mortality rate is higher, which may be compensated for by better adult survival (reviewed in Chamberlain *et al.* 2009; Seress & Liker, 2015; Biard *et al.* 2017). Thus, urbanization may change the relative benefits of male and female offspring, resulting in biased brood sex ratio. In urban environments, reduced availability of natural food sources like arthropods during brood-rearing (see e.g. Seress *et al.* 2018) may have a stronger negative effect on the faster-growing and larger offspring, making the smaller sex more profitable for parents (for similar effects in non-urbanization context, see Rosivall *et al.* 2010). Furthermore, competition for arthropod food may continue after fledging and might be stronger in urban habitats with unfavourable local conditions than in forests, which predicts that parental investment should be biased towards the more-dispersing sex (Julliard, 2000). Thus, studying offspring sex ratios may contribute to a better understanding of how animals adapt to urban environments. However, our knowledge regarding sex ratio adjustment in urban environments is still very limited (Dhondt 1970; Rejt *et al.* 2005; Bonderud *et al.* 2017).

Beside environmental conditions, parental quality is another factor that can influence future reproductive success of male and female offspring, and thus may also affect the brood sex ratio. On the one hand, the "mate attractiveness hypothesis" (Burley, 1981, 1986) states that females mating with males with attractive heritable traits should produce more sons than those who mate with unattractive males, because the formers' sons will be more desirable for females and can achieve higher breeding success (e.g. West *et al.* 2000; Komdeur & Pen, 2002; Yamaguchi *et al.* 2004; reviewed in Booksmythe *et al.* 2017). Larger body size (e.g. as indicated by tarsus length in great tits: Yamaguchi *et al.* 2004) may be one of these attractive heritable male traits. On the other hand, parents of larger body size or in better condition may provide higher quality parental care, which can also influence parents' decision on optimal sex allocation. This latter idea predicts that higher-quality parents who can provide adequate

care under unfavorable conditions (e.g. can provide more and better prey items to the nestlings) will produce more offspring of the more vulnerable sex than lower-quality parents. This, again, predicts an overproduction of the less sensitive sex in urban broods, because body size, condition, and individual quality is often reduced in urban adults (reviewed in e.g. Seress & Liker, 2015).

In this study, we investigated the effects of urbanization on brood sex ratio in great tits, a passerine bird that occupies a wide range of habitats (Burfield & van Bommel, 2004). Great tits are successful urban colonizers, but in cities they often show reduced clutch size, lower nestling mass and fledging success compared to forest areas (Horak, 1993; Chamberlain et al. 2009; Bailly et al. 2015; Seress et al. 2018), likely because of the lower availability of natural prey as nestling food in urban habitats (Seress et al. 2018). In this species, an earlier study found signs of facultative sex ratio adjustment, as primary sex ratios varied with date and clutch size (Lessells et al. 1996). Other studies suggest that different sensitivity of the sexes to habitat quality can also affect the brood sex ratio in this species. For example, Bouvier *et al.* (2016) found that the sex ratio of fledglings was more biased towards females in orchards with high levels of pesticide treatments (hence reduced food availability) compared to moderately treated or organic gardens. Similarly, breeding territory quality also may predict brood sex ratio in woodland great tits: Stauss *et al.* (2005) found that in deciduous forests, where caterpillars (the preferred nestling food) were abundant, broods were more male-biased than in coniferous forests that had reduced caterpillar availability. However, none of the earlier studies investigated habitat-related effects on offspring sex ratios in great tits in an urbanization context. Furthermore, the earlier studies investigated only the fledgling sex ratio (which can be changed by parental adjustment or sex-dependent mortality) and not the primary sex ratio (i.e. sex ratio adjustment by parents).

In great tits male offspring are larger and may be more sensitive to poor environmental conditions (Tschorren et al. 2003), whereas females disperse further and thereby may escape more successfully from unfavourable local conditions (Andreu & Barba 2006). So based on the aforementioned results, we predicted that great tits would produce more female-biased broods in the food-limited urban habitats than in natural forests where nestling food is abundant. We tested this prediction using breeding data from three years of monitoring four populations, two in cities and two in nearby deciduous woodlands. We investigated both the primary sex ratio (i.e. sex ratio at egg laying) and fledgling sex ratio, and we took into account other factors that may influence brood sex ratios, including laying date and, as proxy for parental quality, parental body size (Kölliker *et al.* 1999; Rosivall *et al.* 2004; Bell, Owens

& Lord 2014). Using data on fledgling body size and nestling survival, we also evaluated whether male offspring are larger and more sensitive (in terms of nestling mortality) than females in our populations.

Materials and Methods

Field methods

We studied great tit populations at two forests and two urban sites in Hungary. Forest sites were located in deciduous woodlands near Szentgál (47°06'39.75"N, 17°41'17.94"E) and in Vilma-puszta (47°05'06.7"N, 17°51'51.4"E), whereas the two urban sites were located in the cities of Veszprém (47°05'17.29"N, 17°54'29.66"E) and Balatonfüred (46°57'30.82"N, 17°53'34.47"E). We collected data at all study sites from 2012 to 2014, with the exception of Balatonfüred, where data collection started in 2013. Nest boxes in the urban habitats were placed mostly in public parks and university campuses; all of these plots were strongly influenced by anthropogenic disturbance (e.g. presence of vehicle traffic and human activity; see Seress et al 2018 for more details on the study sites). We monitored the nest boxes at least twice a week from March to early July to record laying date of the first egg, clutch size, hatching dates, and the number of nestlings (detailed in Seress et al. 2017). We ringed all nestlings just before fledging (at 14-16 days of age, day 1 being the hatching day of the first-hatching nestlings) and measured the length of their left tarsus to the nearest 0.1 mm and their right wing (the flattened maximum wing chord, from the carpus to the tip of the longest primary; Svensson, 1992) to the nearest mm, and recorded their body mass (to the nearest 0.1 g using Pesola spring balance). We also took a small drop of blood (ca. 25 µl) from the brachial vein. In 2013-2014, we collected unhatched eggs (that did not hatch for at least 5 days after the first chick of the same brood hatched) and a small tissue sample (e.g. feather, toes) from chicks found dead in the nest during nest box checking throughout the brood rearing period. We stored all samples either in Queen's lysis solution or in 96% ethanol at 4°C until further analysis. We captured adult birds on their nests during brood rearing and ringed each bird with a unique combination of a numbered metal ring and three plastic colour rings for individual identification (Seress et al. 2017). To increase the number of individually identified birds in our populations, we also ringed adult great tits outside of the breeding season (from late September to early February) at the four study sites using mist-nets. Thus, parents of the broods included in our analyses were identified either by capturing them during brood rearing or by observing their colour ring combinations from video recordings filmed with concealed nest cameras (see Seress et al. 2017 for details). On these video samples we

considered a colour-ringed individual to be a parent bird if it was recorded to enter the nest box with food at least once. For measuring and sampling adult birds, we followed the same protocol described above for fledglings.

Laboratory methods

Laboratory methods are described in section 2.2. Out of 44 unhatched eggs, we found 30 infertile eggs. We preserved the embryos from the 14 fertile eggs in 96% ethanol. We then extracted a small sample of tissue from the embryos and the further DNA isolation steps were similar to the methods we used for blood and other tissue samples. All embryos were successfully sexed. We were also able to successfully extract DNA from all of the tissue samples of the dead nestlings.

We analysed 126 broods (14 from 2012, 52 from 2013, and 60 from 2014) where we had blood or other tissue samples from nearly all offspring (i.e. missing tissue sample from no more than 3 dead offspring per brood). We had 79 broods (6 from 2012, 34 from 2013, and 39 from 2014) where we were able to take DNA samples from all offspring (both dead and fledged) and thereby we could calculate the primary sex ratio (i.e. at egg laying). The 6 broods from 2012 that we could include in the primary sex ratio analyses were nests where all laid eggs had become successful fledglings (i.e. there were no unhatched eggs or dead nestlings). In the remaining broods we could estimate only the fledgling sex ratio (i.e. at the age of ringing, at 14-16 days). We aimed to sample both the first and second annual broods at each study site. We categorized each brood as the first annual breeding attempt of a pair if it was initiated before the date of the first egg laid in the earliest identified second clutch in that year at that study site (i.e. clutch by a colour-ringed female that had already successfully fledged at least one young in that year). Broods initiated after this date were categorized as second annual breeding attempts. Our sample size is inherently unbalanced, because the number of available broods differed between sites and years, and changed over the season (i.e. there were fewer second broods than first broods). For the 126 broods, we were able to identify 240 parents, out of which 111 fathers and 118 mothers were measured as adults (the remaining 11 birds were only measured and ringed as nestlings in the previous year); in total, we had 105 broods where both parents were identified and measured.

Statistical analyses

We calculated primary and fledgling sex ratios as number of males divided by the total number of offspring/nestlings. Primary sex ratio means the sex ratio of all offspring (embryos, dead chicks, and chicks that reached the fledging age) in complete broods, whereas fledgling sex ratio means the sex ratio of nestlings that reached the fledging age (without embryos or

dead chicks). We analysed the data from the first and second annual broods together and used the laying date as a covariate in all analyses. We calculated laying date in two alternative ways, and used these two variables in two alternative sets of models. First, we used laying date as the absolute number of days since 1 January until the laying of the first egg in the brood (Julian day). This variable reflects brood value, as offspring fledging later in the season have less time for post-fledging growth before winter. Second, to test the specific effect of timing within the breeding season in each year at each study site, we used mean-centered laying date, subtracting the mean of the respective site and year from each brood's laying date. This variable captures a different aspect of the date effect, as the start of the breeding season varies among sites and years, and the relative timing of broods may affect their food availability (Seress et al. 2018). In the main text, we present the results using the former date variable; see Appendix A/2 for results with the latter date variable (Table A/2.3).

To test whether the primary and fledgling sex ratios differed between study sites, we built generalized linear mixed-effects models with binomial error distribution and “logit” link function (function `glmmPQL` in package `MASS`; Ripley et al. 2013). The full models contained study site, year, laying date (either Julian day or the mean-centered laying date), tarsus length of the father, and tarsus length of the mother as fixed effects and brood ID nested in pair ID as random factors. We also tested the interaction between study site and parents' tarsus length, but it was non-significant in all models ($P > 0.08$), so we present all model results without these interactions to facilitate easier interpretation of the main effects. Note that we did not include other parental body size variables (i.e. wing length, body mass) as predictors of brood sex ratio, because these traits can change considerably throughout the year and in many cases parents' size data were collected outside of their breeding period (see Field methods above). To increase our sample size, we repeated these analyses after excluding parents' tarsus length from the model, because we had data on both parents' tarsus length only in a subset of broods (see Appendix A/2, Table A/2.1 for sample sizes). Henceforward we refer to these analyses as "reduced models". Furthermore, to assess if our results were affected by imbalanced sample sizes due to the different frequency of second annual broods at the four sites, we repeated our sex ratio analyses after excluding the second broods.

To statistically compare the sex ratios between the two habitat types (urban sites vs. forest sites) we calculated linear contrasts from the full and reduced models. These linear contrasts were pre-planned comparisons between the two urban sites vs. the two forest sites (see also chapter 4 & 6, and Vincze *et al.* (2019) for the same approach to compare habitat types by pre-planned linear contrasts and for additional details of the method). Each linear

contrast was back-transformed from the log-scale to provide the odds ratio (OR, i.e. the proportional difference of the odds of an offspring being male between urban and forest broods) with 95% confidence interval (CI). For the linear contrasts, we used the “emmeans” function (emmeans package in R; Lenth 2018).

To investigate sexual size dimorphism in fledglings (measured at ringing, 14-16 days post-hatching; day of hatching = day 1), we used linear mixed-effects models (function `lmer` in package `lme4`; Bates, Maechler & Bolker 2011). We built three separate models in which the response variables were the wing length, tarsus length or body mass of individual fledglings, respectively. In these three models the fixed effects were study site, year, laying date (Julian day only) and sex of the fledgling, while brood ID nested in pair ID and crossed with measurer ID were included as random factors. To test if body size differences between male and female fledglings were different at the four study sites, we added the two-way interaction between sex and study site to these models. Any random variation among broods (including any difference in age) was taken into account by including brood ID as a random factor. We did not include fledgling age at ringing into the model because it varied in a very narrow range (14-16 days); note that Seress et al. (2018) found no significant effect of fledgling age (within the same age interval) on body mass in the same populations between 2013 and 2016.

To test for sex-dependent offspring survival, we analysed the effect of offspring sex on the probability of mortality to fledging. We used a generalized linear mixed-effects model with binomial error distribution and “logit” link function `glmmPQL` in package `MASS` (Ripley et al. 2013). The response variable was the status of offspring as alive (survived to day 14-16) or dead (unhatched eggs and dead chicks), the fixed effect was the sex of the offspring, and the model also included brood ID nested within pair ID as random factors. Because offspring mortality was very rare, especially in forest sites (see Results), we did not investigate whether the sex difference in mortality differed between habitats. All of the tested variables showed acceptable level of multicollinearity, because the variance inflation factor (VIF) varied from 1.04 to 1.22 in all of the models. All analyses were done using R version 3.4.2. (R Core Team 2017).

Results

In our sample, primary sex ratio was overall 0.493, whereas fledgling sex ratio was 0.514 (for sample sizes see Appendix Table A/2.1). For both primary and fledgling sex ratio, none of the tested predictors had significant effects either in the full model (see model estimates in

the Appendix in Tables A/2.2 & A/2.3) or in the reduced model (Table 5.1). Primary sex ratio was statistically close to parity at every study site (estimated mean \pm SE, Veszprém city: 0.55 ± 0.248 ; Balatonfüred city: 0.46 ± 0.390 ; Vilma-puszta forest: 0.46 ± 0.256 ; Szentgál forest: 0.48 ± 0.246 ; the 95% CI includes 0.5 for all sites, see Figure 5.1a) and did not differ significantly between urban and forest sites (Table 5.1). Fledgling sex ratio also did not deviate significantly from parity at any of the four sites (Veszprém city: 0.60 ± 0.171 ; Balatonfüred city: 0.51 ± 0.483 ; Vilma-puszta forest: 0.51 ± 0.265 ; Szentgál forest: 0.52 ± 0.248 ; the 95% CI includes 0.5 for all sites, see Figure 5.1b), and there was no significant difference between urban and forest habitats (Table 5.2). These results were qualitatively identical when we eliminated the second annual broods from the models (see model estimates in Tables A/2.4 & A/2.5).

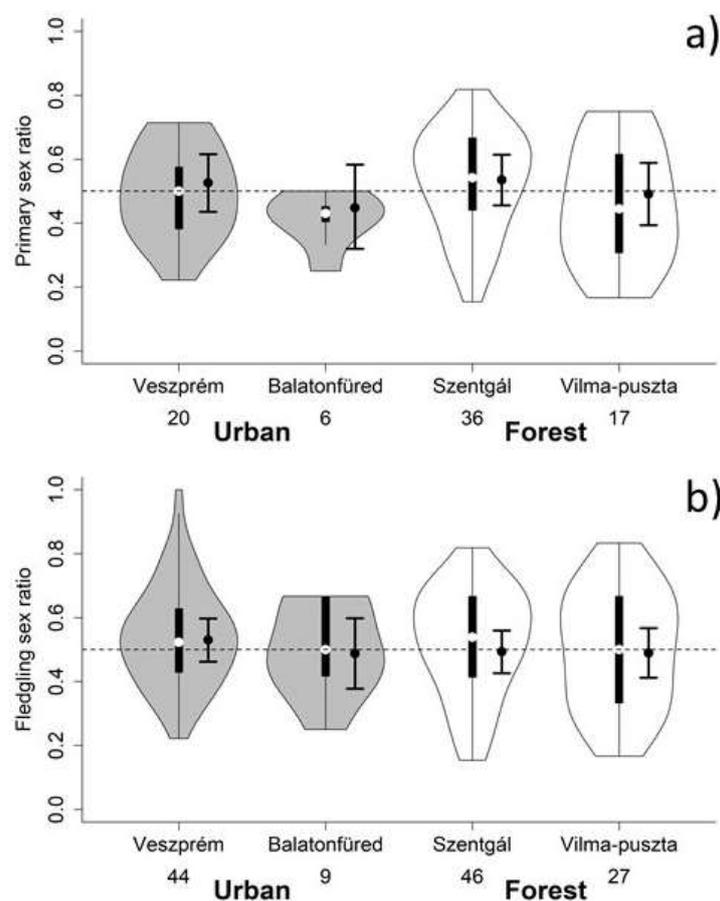
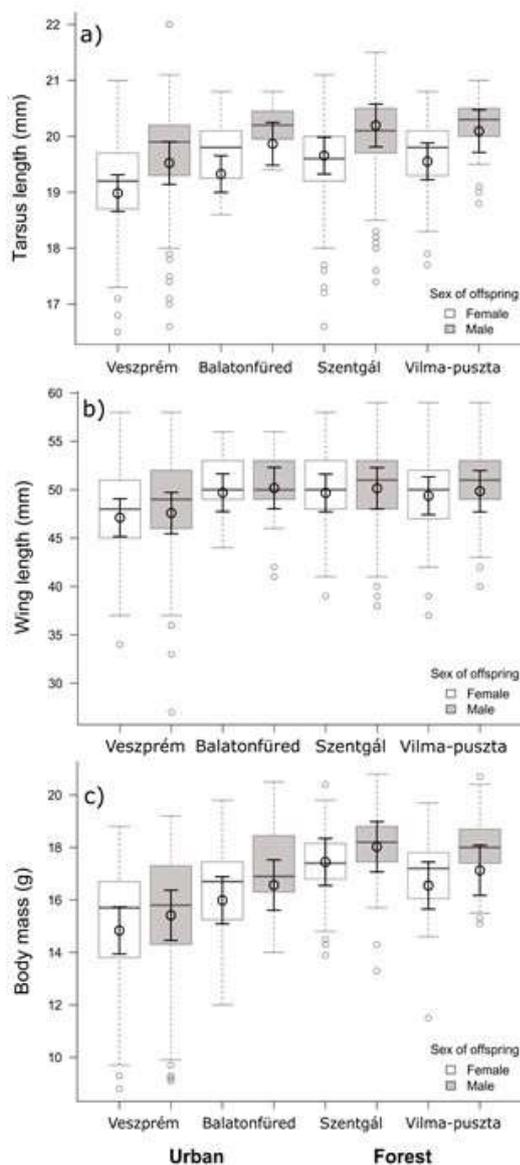


Figure 5.1. Violin plot of the distribution of a) primary sex ratio and b) fledgling sex ratio (proportion of males) in broods at urban and forest study sites (first and second annual broods pooled). Each plots show the median (indicated by the small, open circle), the first through the third interquartile range (the thick, solid vertical band), and estimator of the density (thin vertical curves) at each site. Numbers below the violin plots refer to the number of broods in each site. Dots and whiskers next to the inner box plots show means and 95% confidence intervals, respectively, both calculated from the model shown in Table 5.1.



Male fledglings had longer wings and tarsi and were heavier than female fledglings (Figure 5.2, Table 5.3). These size differences between sexes were independent from the study site (interactions between the sex of the nestlings and study site were non-significant, Table 5.3). None of the body size parameters varied significantly with laying date or among years (Table A/2.6).

Figure 5.2. Body size (a: tarsus length, b: wing length, and c: body mass) of male and female fledglings at the study sites. Box plots show the median, lower and upper quartiles and the whiskers represent data within the $1.5 \times$ interquartile range. The error bars show the mean \pm SE values estimated from the linear mixed models in Table A/2.3. Details on parameter estimates for sex and site effects are provided in Table A/2.6.

In our sample, 10 males and 4 females from 10 broods died in the egg, and 7 male and 6 female nestlings from 9 broods died before ringing. The highest number of dead offspring was found in Veszprém ($n=17$), whereas at the other sites mortality was very low (Balatonfüred: $n=5$, Szentgál: $n=3$, Vilma-pusztta: $n=2$). The sex ratio of dead offspring was 0.63 (0.59 in cities and 0.80 in forests); the proportional difference of the odds of mortality did not differ significantly between males and females (OR= 1.50, CI= 0.91 – 2.47, $P=0.411$).

Table 5.1. Primary and fledgling sex ratio of great tits in relation to study site, year, and laying date (Julian day, first and second annual broods pooled). Effects are presented as analysis of deviance tables with type- 2 sums of squares for the reduced generalized mixed-effects models; n= 79 and 126 for primary and fledgling sex ratios, respectively.

	χ^2	df	<i>P</i>
Primary sex ratio			
Sites	2.040	3	0.564
Years	0.036	2	0.982
Laying date	1.655	1	0.198
Fledgling sex ratio			
Sites	1.707	3	0.635
Years	0.430	2	0.807
Laying date	2.563	1	0.109

Table 5.2. Differences (pre-planned linear contrasts) in primary and fledgling sex ratios between urban and forest habitats, shown as odds ratio (OR; proportional difference of the odds of an offspring being a male at urban sites compared to forests).

	OR [95%CI]	df	t	<i>P</i>
Primary sex ratio				
Full model	0.87 [0.71;1.06]	53	-0.697	0.489
Reduced model	0.90 [0.75;1.09]	72	-0.549	0.584
Fledgling sex ratio				
Full model	1.04 [0.88;1.23]	96	0.236	0.814
Reduced model	1.07 [0.92;1.25]	104	0.473	0.637

Discussion

Contrary to our prediction that great tit parents may overproduce daughters in food-limited urban habitats, we found that neither the primary nor the fledgling sex ratios differed significantly between urban and forest study sites. We consider these results robust, because we collected data over three breeding seasons at four study sites (two urban, two forest), and excluding the second annual broods did not change our results qualitatively (Tables S4 & S5). Our results differ from the findings of two other studies comparing great tits' offspring sex ratios between habitats of different quality. In one of these earlier studies, where the sexing of nestlings was based on visual cues (Dhondt 1970), more male offspring were found in urban

compared to suburban or woodland habitat before fledging. In the other study, Bouvier et al. (2016) found more male nestlings in organic orchards with less pesticide use (that likely represent better habitat quality) than in orchards cultivated by using large amounts of pesticide. The reason for the varying results among these studies is unclear. Notably, the aforementioned studies showed information only about fledgling sex ratio, so to our knowledge our study is the first that compare primary sex ratio between urban and forest habitats in great tits.

With the available information, we can only speculate why we did not find sex ratio adjustment in urban habitats. First, it is possible that in our study populations male or female offspring did not differ in the associated costs of producing and raising them until independence. However, 14-16 days old male fledglings were significantly heavier (by 3.6%) and had slightly longer tarsi (by 2.5%) and wings (by 2%) compared to their female siblings, regardless of habitat type. These results suggest that male nestlings require more parental provisioning during their development than females, although we do not know the extent (and hence the associated additional costs) of such extra provisioning. Apparently, parents were able to meet this requirement in both habitats, because the size difference between male and female fledglings was similar in all study sites, and we did not find any evidence for sex-related mortality. This seems to contradict earlier studies in other great tit populations, which reported either male-biased sex ratio in unhatched eggs (Cichoń, Sendecka & Gustafsson 2005) or higher mortality in females before fledging (e.g. Smith et al. 1989; Lessells et al. 1996), and in some cases growth of females was more severely affected by poor condition in tit species (Oddie 2000; Nomi, Yuta & Koizumi 2018). To better understand these conflicting results, we need to have more data on the sex-specific mortality rates before and after hatching from our study populations and also on the environmental factors and parental quality variables that can influence embryo and nestling survival. For example, it is possible that the increased resource requirement of male offspring induces male-biased mortality only under unusually poor conditions, such as harsh weather, high prevalence of parasites or disease, or extremely low food supply (Tschirren et al. 2003).

Given that the larger size of male fledglings suggests higher parental cost, a potential explanation for the lack of sex ratio adjustment is that there may be some unknown cost to producing female offspring that cancels out the differences in the pay-off between the sexes. For example, it is possible that survival chances are lower after fledging for females than for males. The most dangerous period in the life of juvenile great tits is the dispersion after fledging, as 47% of juveniles great tits and coal tits (*Parus ater*) died during the first 20 days

after fledging in a research (Naef-Daenzer, Widmer & Nuber 2001). Female great tits disperse farther than males (Andreu & Barba 2006), which may mean higher risk of mortality for females, especially in urban habitats where the potential breeding and feeding sites are more fragmented by built-up areas and roads with heavy traffic. Furthermore, survival during autumn and winter may also differ between the sexes in a habitat-dependent manner. In urban areas, seeds and other food in artificial feeders can increase the chance of survival (Marzluff 2017), but competition at these feeders can be stronger than at natural feeding sites such as tree canopies. At these feeders, social rank can limit access to food, because subordinate individuals may be attacked by dominant ones and therefore get less food. In great tits, males are more often dominant than females, especially in juveniles (Barluenga, Barbosa & Moreno 2000; Dingemanse & De Goede 2004). These sex differences in great tit life history may generate female-biased mortality, especially in urban habitats. However, the only published study that compared the sex-specific survival of great tits in both urban and rural habitats found higher adult female than male survival in both habitats, and yearling females outnumbered yearling males in next year in breeding season (Hörak & Lebreton 1998).

We found remarkably high variance of sex ratios among individual broods in both habitat types (primary sex ratio, range in urban habitat: 0.22 – 0.71, in forest habitat: 0.15 – 0.82; fledgling sex ratio, urban: 0.22 – 1.00, forest: 0.15 – 0.84). This variance in our data was not explained by laying date and the parents' tarsus length, representing proxies for seasonal environmental changes and for parental quality, respectively. One interpretation of this high variance is that parents vary in their investment into their offspring's sex, but their allocation is determined by factors which we did not investigate. For example, Lessells *et al.* (1996) reported that the proportion of male offspring increased with hatching asynchrony in great tits. Furthermore, in the same populations and breeding seasons as in the present study, we found that the number of extra-pair offspring was higher in urban habitats than in forests (chapter 6), which might influence sex ratio adjustment. The other possible interpretation of our findings is that the observed variance in brood sex ratios is largely random, with no facultative sex ratio adjustment going on (Ewen *et al.* 2004). For example, in urban areas, where the environmental changes may be rapid and unpredictable, sex ratio manipulation might not be a profitable strategy, as it may be difficult for parents to predict the conditions their offspring will find themselves in. So far, there have been very few studies on great tit primary sex ratios, and their results provided little if any evidence that the observed variation among nests is adaptive (Lessells *et al.* 1996; Kabasakal & Albayrak 2012).

Taking our results together with the small number of previous findings, the role of facultative sex ratio adjustment in birds' adaptation to urban life is not yet clear. Further studies are needed to better understand the prevalence and drivers of offspring sex ratio in an urbanization context. Research is needed also on the sex-dependent effects of urbanization on life-history traits and thus the fitness pay-offs of producing sons and daughters along the urbanization gradient, including sex-related post-fledging survival and future breeding success of male and female offspring in different habitat types. Furthermore, urbanization may interact with other anthropogenic influences such as climate change, potentially resulting in complex effects on sex ratios if males and females differ in their sensitivities to these various challenges.

Table 5.3: Results of the analyses of body size parameters of nestlings in relation to their sex, study site, years and laying date. Effects are presented as analysis of deviance tables with type- 2 sums of squares for the reduced generalized mixed-effects models. Nestlings of first (n= 952) and second annual broods (n= 200) were pooled in the analyses.

	Mean squares	df	F	<i>P</i>
Wing length				
Sex	35.649	1,1032	4.023	0.045
Site	39.238	3,98	4.427	0.006
Years	4.719	2,118	0.5324	0.588
Laying date	0.468	1,77	0.053	0.819
Sex × Site	12.006	3,1035	1.355	0.255
Tarsus length				
Sex	34.540	1,1035	148.430	<0.001
Site	2.105	3,115	9.046	<0.001
Years	0.244	2,129	1.049	0.353
Laying date	0.008	1,104	0.034	0.854
Sex × Site	0.230	3,1038	0.990	0.397
Body mass				
Sex	59.196	1,1024	66.219	<0.001
Site	16.296	3,101	18.229	<0.001
Years	0.891	2,122	0.996	0.372
Laying date	0.005	1,101	0.005	0.943
Sex × Site	0.801	3,1025	0.896	0.443

6. Comparing the frequency of extra-pair paternity between urban and forest great tits (*Parus major*, L.)³.

Introduction

Urban animals often face different ecological and environmental conditions than conspecifics living in more natural habitats (Gil & Brumm 2013; Rodewald & Gehrt 2014; Seress & Liker 2015). In birds, many species successfully colonized urban areas worldwide, and urban individuals have to cope with several anthropogenic environmental changes such as noise, light and chemical pollution (Seress & Liker 2015), habitat fragmentation (Crooks, Suarez & Bolger 2004), and ecological challenges such as higher population densities (Moller *et al.* 2012) and lower availability of natural food (Seress *et al.* 2018). These differences between urban and natural habitats may alter the costs and benefits of birds' reproductive decisions thereby affecting their behaviour. In line with this, urban birds typically show altered reproductive biology including advanced laying dates, reduced brood sizes, higher nest-failure rates and smaller nestlings (Bailly *et al.* 2016; Seress *et al.* 2018) compared to their non-urban conspecifics.

A widespread reproductive behaviour among pair-bonding species is the pursuit of extra-pair fertilizations (EPF), which are found in approximately 90% of socially monogamous bird species (Griffith, Owens & Thuman 2002). EPF can increase the number of offspring for males, and it may grant genetic benefits to females by improving fertilization success and/or offspring quality (Griffith *et al.* 2002). These adaptive functions of EPF have been supported by empirical data, although not unequivocally (Hsu 2014; Arct, Drobniak & Cichoń 2015). The frequency of EPF can also be influenced by the spatiotemporal distribution of mating opportunities (Schlicht, Valcu & Kempenaers 2014; García-Navas *et al.* 2015) and physical environmental factors such as night lighting and anthropogenic noise (Kempenaers *et al.* 2010; Halfwerk *et al.* 2011). Many of these potential impacts on extra-pair mating behaviour may be affected by the altered ecological conditions of urban habitats. However, very few studies have compared EPF between birds breeding in urban and natural habitats (Moore *et al.* 2012; Rodriguez-Martínez *et al.* 2014, Bonderud *et al.* 2018), and it remains unclear whether and how extra-pair behaviour varies with habitat urbanization.

³ This chapter is based on the article **Pipoly, I.**, Szabó, K., Bókony, V., Preiszner, B., Seress, G., Vincze, E., Schroeder, J., Liker, A. (2019): Higher frequency of extra-pair offspring in urban than forest broods of great tits (*Parus major*), *Frontiers in Ecology and Evolution*, DOI: 10.3389/fevo.2019.00229

The aim of our study was to test whether the frequency of extra-pair offspring (EPO; i.e. offspring that are not genetically related to the social partner of the female) within and across broods differs between urban and non-urban great tits (*Parus major*), a common and successfully urbanized passerine species with relatively high EPF rates (García-Navas *et al.* 2015). To test this we used a data set from two urban and two forest populations from three consecutive breeding seasons.

Methods

We studied great tits breeding in nestboxes at two urban and two forest sites in Hungary from 2012 to 2014 (see detailed field methods in section 2.1). We selected 86 first annual broods of marked parents and conducted multi-locus genotyping (see molecular methods in section 2.2). We identified an offspring as EPO if it mismatched the alleles of the social father on at least two loci but it had no mismatch with the maternal alleles. We successfully genotyped all blood samples ($n = 978$ in total, 159 adults and 819 nestlings) and all tissue samples from dead nestlings ($n = 17$). We found 16 embryos in the 46 unhatched eggs, and 15 (93.8 %) embryos were successfully genotyped. There were nine parents with reconstructed genotypes (Jones *et al.* 2010) and 14 parents with more than one of their broods genotyped in different years ($n = 21$ broods). The 5 microsatellite markers we chose were highly variable (see Table 2.2.1 above for diversity indices). Both expected and observed heterozygosities for the 5 loci averaged 0.88 (SD = 0.03). The probability of identity when siblings were present was 2.99×10^{-3} for the 5 loci combined and 1.42×10^{-4} for the 8 loci combined. Using MICROCHECKER 2.2.2 (Van Oosterhout *et al.* 2004) we did not find evidence for large-allele dropout and genotyping errors due to stutter bands at any of the 8 loci; null alleles may have been present at one locus (PmaGAn27). Using GENPOP 4.0 (Rousset 2008) we detected no departure from Hardy-Weinberg equilibrium, but significant linkage disequilibrium for 3 pairs of loci (PmaTGAn59 with PmaTAGAn89, PmaTGAn33 with PmaGAn27 and PmaTGAn54; $p < 0.001$). To further validate our 5-loci marker set, we conducted parentage analysis with CERVUS 3.0 (Kalinowski, Taper & Marshall 2007) using the data of 82 candidate fathers and 58 offspring that had no mismatch with their social father's genotype, and providing the mothers' genotype. For each offspring tested, its social father received positive LOD score (i.e. the sum of the log-likelihood ratios at each locus), meaning that this male was more likely to be the genetic father than the other candidate fathers were. When more than one candidate fathers had positive LOD scores ($n = 26$ offspring), the social father always ranked first, i.e. had the highest LOD score. Thus, our 5-loci marker set proved

reliable and efficient for discriminating between within-pair offspring (WPO) and extra-pair offspring (EPO).

The number of paternally mismatched loci per offspring ranged between 2 and 7. We could not identify the genetic father of the majority (47 out of 60) of EPO because we did not have DNA samples from all males in each population. Altogether 159 parents (80 males and 79 females) and 851 offspring (n=819 live chicks, n=17 dead chicks, n=15 embryos in unhatched eggs) were genotyped in 86 families. There were seven males and seven females that each had two of their broods in different years genotyped (n = 21 broods). Out of the 86 broods, 50 were complete broods where all offspring (i.e. all the laid eggs) were genotyped either because every egg hatched and reached the age of blood sampling or because every unhatched egg and/or dead nestling was sampled. For 13 additional broods, we found no embryos in the collected unhatched eggs (n = 30 eggs), so we assumed that these eggs were infertile and we treated these broods as complete. The remaining 23 broods were incomplete because some unhatched eggs (n = 12 broods) or dead nestlings (n = 8 broods) or both (n = 3 broods) disappeared from the nest before we could sample them. From the 17 dead nestlings that we genotyped, two were identified as EPO, and there were additional EPO in the same broods among the alive EPO. None of the genotyped 15 embryos were identified as EPO. We found EPO in 62% of the incomplete urban broods (8 / 13 broods) and 44% of the complete urban broods (14 / 32 broods). In forest broods, we found EPO in 20% of incomplete broods (2 / 10 broods) and 26% of the complete broods (8 / 31 broods). The proportion of incomplete broods did not differ between urban (13 / 45) and forest (10 / 41) broods (Fisher's exact test: odds ratio: 1.26, p = 0.808), and the frequency of EPO in incomplete broods was not different from the frequency of EPO in complete broods (Fisher's exact test, urban broods: odds ratio: 2.02, p = 0.337, forest broods: odds ratio: 0.72, p > 0.99). Thus, incomplete broods are unlikely to introduce bias in our data, and considering the missing unsampled offspring in incomplete broods as WPO makes our estimates of EPO occurrence conservative, so we analysed complete and incomplete broods together.

Statistical analyses

We tested the difference in EPO frequencies between urban and forest broods by Generalised Estimation Equations (GEE) models to accommodate non-normal error distributions, and we allowed for the non-independence of those few broods that had at least one parent in common using the 'exchangeable correlation' association structure (Zuur *et al.* 2009). We investigated three response variables. (1) For testing the difference in the proportion of broods that contained EPO, the response was a binary variable of EPO presence

in a brood (EPO present / absent), and a GEE model was constructed with binomial error structure and logit link function. As clutch size varies between urban and non-urban birds (Seress *et al.* 2018), we compared the quantity of EPO between urban and forest broods in two ways, analyzing (2) the number of EPO in a brood using a GEE model with Poisson distribution and log link function, and (3) the proportion of EPO within the brood, the latter calculated as $EPO / (EPO + WPO)$, using a GEE model with binomial error structure and logit link function. The model for EPO occurrence contained all 86 broods, while the models for EPO number and EPO proportion contained only those 32 broods where at least one EPO occurred. We chose this subset in the latter models to avoid zero inflation, and to explicitly address the question whether urban and forest parents that engage in EPF differ in their allocation into extra-pair offspring. We included the main effects of study site (4 sites) and year (3 years) in all models (note that testing the site \times year interaction in this model was not feasible because we have no data for Balatonfüred from 2012). As data from 2012 was lacking for one urban site, and tissue samples were not collected in this first study year, we repeated the analyses with the data only from 2013 and 2014. In these analyses, sample size was 72 broods for EPO occurrence, and 27 broods for the number and proportion of EPO (where at least one EPO occurred). To statistically compare the two habitat types, we calculated a linear contrast from each of the three GEE model's estimates (i.e. the difference between the two urban sites and the two forest sites, on log scale). Our design corresponds to an experiment with 4 treatment groups (i.e. 2 control groups and 2 treatment groups) and our linear contrasts are pre-planned comparisons among those groups (Ruxton & Beauchamp 2008). Because the GEE model estimates were on log scale, each linear contrast was back-transformed from the log-scale to provide the odds ratio (i.e. proportional difference of the odds of having at least one EPO, or a chick being EPO, between urban and forest broods) for EPO presence and EPO proportion and the incidence rate ratio (i.e. proportional difference of EPO frequency between urban and forest broods) for EPO number. All analyses were implemented in the R 3.1.1 software environment (R Core Team 2014), using packages “geepack” (Højsgaard, Halekoh & Yan 2006), “emmeans” (Lenth 2018) and “multcomp” (Hothorn, Bretz & Westfall 2008).

Results

There were more broods containing EPO in urban than forest habitat (Figure 6.1, Table 6.1) in all three study years (urban vs. forest 2012: 40.00 vs. 33.33 %; 2013: 39.13 vs. 6.67 %; 2014: 64.71 vs. 35.29 %). When we controlled for non-independence of parents and

differences among years in a GEE model (Table 6.2), EPO occurrence was significantly higher in urban than forest broods (odds ratio calculated from urban-forest linear contrast = 5.77, 95 % confidence interval (CI): 1.72 – 19.4, $p = 0.005$).

The number and proportion of EPO was slightly higher in urban broods than in forests when we considered all broods (Table 6.1), but when we considered only the broods with at least one EPO there was no significant difference between urban and forest habitats in the number of EPO per brood (Table 6.1, Figure 6.2, Table 6.2, proportional difference between urban and forest broods from linear contrast = 1.175, 95 % CI: 0.667 – 2.067, $p = 0.58$) and the proportion of EPO per brood (Table 6.1, Figure 6.3, Table 6.2, odds ratio = 1.65, 95 % CI: 0.793 – 3.43 from urban-forest linear contrast, $p = 0.18$). The results for all three variables of interest remained qualitatively the same when we omitted data from 2012, i.e. the year when we did not have data for one urban site and did not collect samples from dead chicks and unhatched eggs (Table 6.3).

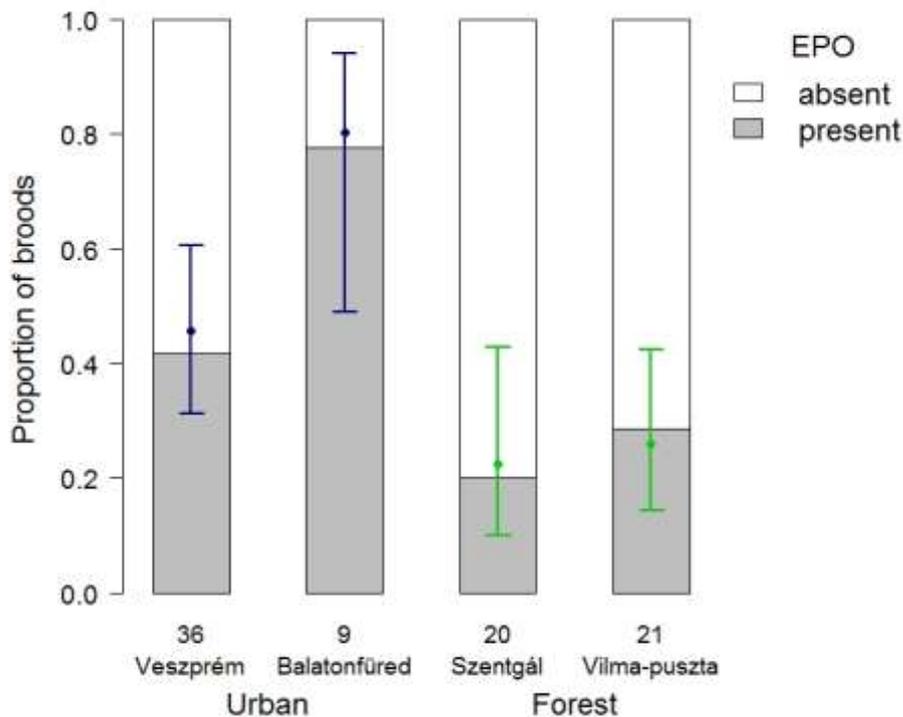


Figure 6.1. Occurrence of extra-pair offspring (EPO) in great tit broods at urban and forest study sites. Numbers below the bars refer to the number of genotyped broods in each site. Dots and whiskers show means and 87% confidence intervals, respectively, both calculated from the GEE model with study sites and years as predictors. Non-overlapping 87% CIs indicate statistically significant difference (i.e. that a 95% CI of the difference excludes zero).

Table 6.1. Occurrence of extra-pair offspring (EPO), i.e. proportion of studied broods that contained at least one EPO, and the percentage of EPO among all nestlings in the two urban and two forest study sites.

	Urban			Forest		
	All urban	Veszprém	Balatonfüred	All forest	Vilma-pusztá	Szentgál
EPO occurrence ¹	48.89 % (45)	41.6 % (36)	77.7 % (9)	24.39% (41)	28.6 % (21)	20.0 % (20)
EPO % within all studied broods ²	10.49% (393)	8.1 % (310)	19.3 % (83)	4.15% (458)	4.4 % (226)	3.9 % (232)
EPO % in EPO-containing broods only ²	20.92% (196)	18.9 % (132)	25.0 % (64)	16.96% (112)	14.7 % (68)	20.5 % (44)

¹ Number of studied broods are given in parentheses

² Number of genotyped chicks are given in parentheses

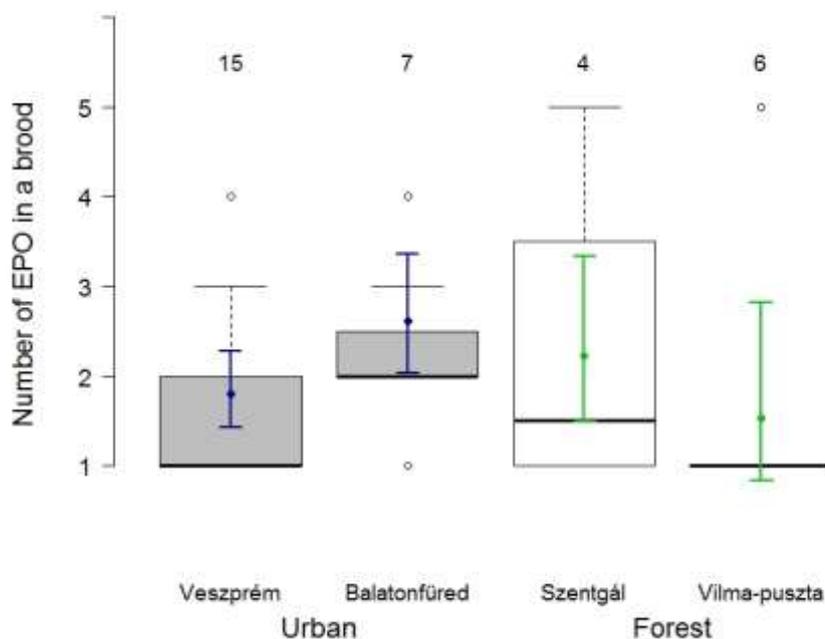


Figure 6.2. Number of extra-pair offspring (EPO) in EPO-containing broods in our study sites. Numbers above the boxes refer to the number of genotyped broods in each site. In each boxplot, the thick middle line and the box shows the median and the interquartile range, respectively; dashed whiskers extend to the most extreme data points within $1.5 \times$ interquartile range from the box, and empty dots represent more extreme data points. Coloured filled dots and solid whiskers show the mean and its 87% confidence interval, respectively, both calculated from the GEE model with study sites and years as predictors. Non-overlapping 87% CIs indicate statistically significant difference (i.e. that a 95% CI of the difference excludes zero). The number of EPO was one in $n=9$ urban and $n=7$ forest broods, two in $n=9$ urban and $n=1$ forest broods. There were $n=2$ urban broods with three EPO, $n=2$ additional urban broods with four EPO, and $n=2$ forest broods with five EPO.

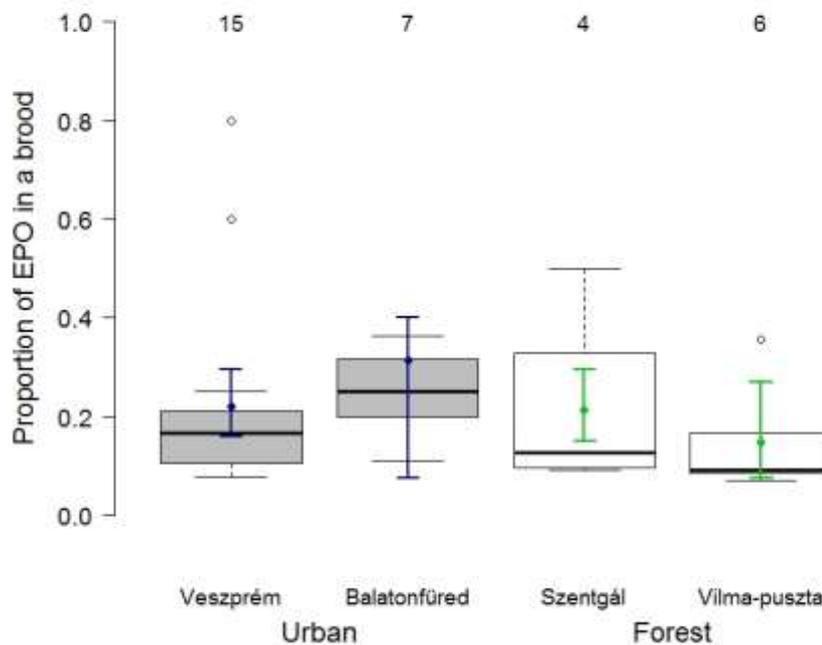


Figure 6.3. Proportion of extra-pair offspring (EPO) in EPO-containing broods in our study sites. Numbers above the boxes refer to the number of genotyped broods in each site. In each boxplot, the thick middle line and the box shows the median and the interquartile range, respectively; dashed whiskers extend to the most extreme data points within $1.5 \times$ interquartile range from the box, and empty dots represent more extreme data points. Coloured filled dots and solid whiskers show the mean and its 87% confidence interval, respectively, both calculated from the GEE model with study sites and years as predictors. Non-overlapping 87% CIs indicate statistically significant difference (i.e. that a 95% CI of the difference excludes zero). The proportion of EPO where at least one EPO occurred ranged from 0.08 to 0.80 in urban broods (median: 0.2) and between 0.07 and 0.50 in forest broods (median: 0.095).

We detected two events of intraspecific brood parasitism in 2014 with 2 out of 9 (Veszprém, urban site) and 3 out of 13 nestlings (Szentgál, forest site) being mismatched with their social mother's genotype on at least two loci. We also found one case of interspecific brood parasitism in one urban nestbox in Veszprém in 2013, where a blue tit (*Cyanistes caeruleus*) nestling was reared successfully in a great tit brood.

Table 6.2. Results of Generalised Estimation Equations (GEE) models for the occurrence, number and proportion of EPO in great tit broods. Study site (4 sites) and year (3 years) were the predictor variables in the models. Exponentially back-transformed parameter estimates (e^b) for the four sites provide the odds of having at least one EPO in a brood, the frequency of EPO in broods that contained EPO, and the odds of each offspring being EPO in broods that contained EPO, in the year 2014. For years 2012 and 2013, e^b provides the difference from 2014, i.e. the odds ratio of having at least one EPO, the proportional difference of the frequency of EPO, and the odds ratio of each offspring being EPO. Parameter estimates (**b**) with their standard errors (**SE**) from the GEE models are also presented, and **n** is the number of genotyped broods in the analyses. Linear contrast for comparing the urban and forest habitat types (presented in Results in the main text) were calculated from the parameter estimates of these models.

Model parameters	EPO occurrence (n=86)				EPO number (n=32)				EPO proportion (n=32)			
	b ± SE	e^b	χ^2	p	b ± SE	e^b	χ^2	p	b ± SE	e^b	χ^2	p
site: Veszprém	0.301 ± 0.466	0.575	0.42	0.519	0.541 ± 0.140	1.720	14.89	< 0.001	-1.589 ± 0.187	0.170	72.33	< 0.001
site: Balatonfüred	1.877 ± 0.916	0.867	4.20	0.040	0.911 ± 0.152	2.490	35.84	< 0.001	-1.110 ± 0.191	0.248	33.74	< 0.001
site: Szentgál	-1.764 ± 0.608	0.318	1.58	0.209	0.753 ± 0.259	2.120	8.41	0.473	-1.626 ± 0.301	0.164	29.25	< 0.001
site: Vilma-puszta	-0.564 ± 0.586	0.362	0.93	0.336	0.377 ± 0.465	1.460	0.66	0.715	-2.073 ± 0.582	0.112	13.12	< 0.001
year: 2012	-0.291 ± 0.715	0.428	0.17	0.684	0.357 ± 0.326	1.430	1.20	0.273	0.948 ± 0.546	0.721	3.02	0.082
year: 2013	-1.142 ± 0.460	0.242	6.18	0.013	-0.209 ± 0.186	0.811	1.25	0.263	0.033 ± 0.272	0.508	0.01	0.905

Table 6.3. Results of Generalised Estimation Equations (GEE) models for the occurrence, number and proportion of EPO in great tit broods when only data for 2013 and 2014 are included in the analyses. Study site (4 sites) and year (2 years) were the predictor variables in the models.

Model parameters	EPO occurrence (n=86)				EPO number (n=32)				EPO proportion (n=32)			
	b ± SE	e^b	χ^2	p	b ± SE	e^b	χ^2	p	b ± SE	e^b	χ^2	p
site: Veszprém	0.409 ± 0.485	0.601	0.71	0.399	0.507 ± 0.097	1.660	27.10	<0.001	-1.658 ± 0.133	0.160	154.76	<0.001
site: Balatonfüred	1.839 ± 0.905	0.863	4.13	0.042	0.896 ± 0.154	2.450	33.94	<0.001	-1.139 ± 0.194	0.243	34.44	<0.001
site: Szentgál	-1.061 ± 0.672	0.257	2.49	0.114	0.342 ± 0.198	1.410	2.98	0.084	-2.048 ± 0.200	0.114	105.24	<0.001
site: Vilma-puszta	-0.400 ± 0.621	0.401	0.42	0.519	0.693 ± 0.433	2.000	2.56	0.109	-1.658 ± 0.478	0.160	12.04	<0.001
year: 2013	-1.095 ± 0.446	0.251	6.02	0.014	-0.171 ± 0.172	0.843	0.99	0.320	0.109 ± 0.260	0.527	0.18	0.673

Discussion

We found that urban broods of great tits contained at least one EPO significantly more often than forest broods, and this pattern was consistent across all three years. This result corroborates findings on other species: EPF tended to be higher in more urbanized areas in Canada geese (*Branta canadensis*) (Moore *et al.* 2012) and an unusually high rate of EPF was registered in urban Cooper's hawks (*Accipiter cooperii*) (Rosenfield *et al.* 2015). However, there was no habitat difference in EPF rate of mountain chickadees (*Poecile gambeli*) (Bonderud *et al.* 2018). On the other hand, in broods where EPO occurred we did not find difference in the number and proportion of EPO between urban and forest habitats. Thus our results suggest that urbanization is associated with increased occurrence of EPF in great tits, although those females that engage in EPF produce similar numbers of EPO in both habitat types. As we examined four populations in comparing the two habitat types, further research will be required to assess the generality of our findings.

Several factors might explain the higher occurrence of EPO in urban populations. First, urban sites may offer more opportunities in space and time, for example through higher breeding density. Urban birds have on average 30 % higher breeding density than rural populations (Moller *et al.* 2012)ler *et al.* 2012) and higher breeding density can increase EPF frequency (Charmantier & Perret 2004; Mayer & Pasinelli 2013). Moreover, urban individuals often have a prolonged diurnal activity due to artificial lighting at night (Dominoni *et al.* 2013), offering more time for females to search for extra-pair males during early dawn (Double & Cockburn 2000; Kempnaers *et al.* 2010; Halfwerk *et al.* 2011). Furthermore, night lighting enables males to start singing earlier and such males are more successful in siring EPO (Silva, Valcu & Kempnaers 2015).

Further, food availability and its seasonal distribution may also contribute to the differences in extra-pair mating behaviour between urban and forest populations. Caterpillars, the main food of great tits during the breeding season, are scarce in cities (Biard *et al.* 2017; Seress *et al.* 2018), so urban males might have to spend more time foraging at the expense of guarding their females during their fertile period, increasing opportunities for EPF in cities. However, previous experimental studies found that food scarcity can either increase (Hoi-leitner *et al.* 1999) or decrease EPF rates in various species (Václav, Hoi & Blomqvist 2003; Kaiser *et al.* 2015). Furthermore, urban habitats lack the massive spring peak of caterpillar abundance that is typical in forests (Seress *et al.* 2018), which might lead to less synchronized breeding in urban populations, further facilitating EPF (Van Dongen & Mulder 2009; García-Navas *et al.* 2015), although an opposite effect of breeding asynchrony is also possible (Stutchbury & Morton 1995; Neudorf 2004).

Also, birds might gain more benefits from EPF in urban habitats than in forests. Urban birds can suffer from a higher risk of inbreeding (Vangestel *et al.* 2011; Rodriguez-Martínez *et al.* 2014) and reduced reproductive success per breeding attempt (Bailly *et al.* 2016; Biard *et al.* 2017; Seress *et al.* 2018), so any benefit gained from fertility insurance and good genes might have more value in urban areas (Reding 2014). Furthermore, urban females may be more motivated to pursue extra-pair partners because they might be more likely to perceive their social mate's quality as low. Individual quality and/or signals like song and plumage ornaments in birds are often relatively poorly developed in urban habitats, including the great tits' yellow plumage coloration (Halfwerk *et al.* 2011; Biard *et al.* 2017), black breast stripe (Senar *et al.* 2014) and song characteristics (Slabbekoorn & Peet 2003). Urban great tit males that sing higher-frequency songs to overcome the noise-induced communication breakdown are cuckolded more often (Halfwerk *et al.* 2011), although another study found no difference in EPF in house sparrows (*Passer domesticus*) between noisy and quiet breeding places (Schroeder *et al.* 2012).

Alternatively, the relationship between urbanization and EPO occurrence might be non-causal. For example, individuals with different behavioural types can differ in their habitat choice (Holtmann *et al.* 2017; Sprau & Dingemanse 2017) and the propensity to engage in EPF can vary between females as part of their personality (Forstmeier 2007; Forstmeier *et al.* 2014; Wolak *et al.* 2018). It is thus possible that a tendency for promiscuity is associated with the behavioural traits that facilitate settlement in cities, such as innovative problem solving and exploratory behaviour (Bókony *et al.* 2017).

Taken together, we found higher frequency of EPO containing broods in cities than in forests in a common, successfully urbanized passerine, the great tit. There are many conceivable mechanisms by which habitat urbanization may influence the frequency of EPF. Understanding how these mechanisms shape avian reproductive behaviours, and why their effects differ between species, will further expand our knowledge on urban behavioural ecology and sexual selection. Furthermore, because EPF can decrease inbreeding and increase genetic diversity, uncovering the genetic mating systems of urban animals and identifying the drivers of urban cuckoldry may aid the conservation of fragmented populations in our urbanizing world.

7. Adult sex ratios are predicted by the genetic sex determination system in tetrapods ⁴

Introduction

Adult sex ratio (ASR, proportion of males) varies widely in nature, ranging from populations that are heavily male-biased to ones composed only of adult females (Trivers 1972; Donald 2007). Birds and schistosome parasites tend to have male-biased ASR, for example, while mammals and copepods usually exhibit female-biased ASR (Székely *et al.* 2014b). Extreme bias occurs among marsupials (Didelphidae and Dasyuridae): males die after the mating season, so there are times when the entire population consists of pregnant females (Cockburn, Scott & Dickman 1985). Understanding the causes and consequences of ASR variation is an important goal in evolutionary biology, population demography and biodiversity conservation because ASR impacts behaviour, breeding systems, and ultimately population fitness (Bessa-Gomes, Legendre & Clobert 2004; Le Galliard *et al.* 2005; Kokko & Jennions 2008; Liker *et al.* 2014; Székely *et al.* 2014a). It is also a significant issue in social sciences, human health and economics, since unbalanced ASRs have been linked to violence, rape, mate choice decisions and spread of diseases like HIV (Griskevicius *et al.* 2012; Schacht, Rauch & Borgerhoff Mulder 2014). The causes of ASR variation in wild populations, however, remain obscure (Wilson 1975; Székely *et al.* 2014b; a).

One factor that could impact the ASR is the genetic sex-determination system (Haldane 1922; Trivers 1972; Donald 2007). Taxa such as mammals and fruit flies have XY sex determination (males are heterogametic), whereas taxa such as birds and butterflies have ZW sex determination (females are heterogametic). Sex-determination systems could affect the ASR in several ways. A skewed ASR might result from an unbalanced sex ratio at birth caused by sex ratio distorters (Burt & Trivers 2008). Alternatively, a biased ASR could develop after birth if sex chromosomes contribute to sex differences in mortality (Haldane 1922; Trivers 1972; Liker & Székely 2005). Differential postnatal mortality is likely to be the main driver of biased ASR in birds and mammals, since birth sex ratios in these classes tend to be balanced (Donald 2007).

⁴ This chapter is based on the research article **Pipoly, I., Bókony, V., Kirkpatrick, M., Donald, P.F., Székely, T., Liker, A.** (2015): The genetic sex-determination system predicts adult sex ratios in tetrapods, *Nature*, 5 527(7576), 91-94

Here we use data from the four major clades of tetrapods (amphibians, reptiles, birds and mammals) to assess whether ASRs, measured by convention as the proportion of males in the population, differ between taxa with XY and ZW sex determination (Figure 7.1). While mammals and birds are fixed for XY and ZW sex determination, respectively, reptiles and amphibians provide particularly attractive opportunities for this study since transitions between sex-determination systems have occurred many times within these clades (Bachtrog *et al.* 2014). We compiled published data on adult sex ratios in wild populations and their sex-determination systems. To control for phylogenetic effects, we used phylogenetic generalized least squares models (PGLS, Pagel 1998) to test for differences in ASRs between XY and ZW taxa, and Pagel's discrete method (PDM, Pagel 1994) to test whether XY and ZW systems are evolutionarily associated with female-biased and male-biased sex ratios, respectively.

Methods

Detailed information about data collection is given in section 2.3, and Appendix A/3 contains additional analyses to validate our methods. We included only species with known GSD system from amphibians (Evans *et al.* 2012; Ashman *et al.* 2014), because GSD is an evolutionarily labile trait in amphibians; species within a genus or even populations within a species can differ in GSD (Miura, Ohtani & Ogata 2012). For reptiles, we included all species for which GSD was known either at the family level, or at the species level if both XY and ZW systems were found in the family (Pokorná & Kratochvíl 2009; Sarre, Ezaz & Georges 2011; Ashman *et al.* 2014). All birds were assigned to ZW, and all mammals to XY sex-determination systems (Ashman *et al.* 2014). When more than one ASR measure was available, we used the total counts of individually marked animals over the study period because this may best approximate the overall ASR. We excluded studies in which the authors explicitly stated or speculated that their data may not represent the population-level ASR, or when the methods were not described in enough detail to assess the reliability of the ASR estimate. When more than one estimate of ASR was available for the same population (e.g. from several yearly counts at the same location) we took their mean weighted by sample size. When more than one independent record was available for a species from different populations or studies, we used their simple mean. To assess the reliability of the amphibian and reptile ASR estimates, we calculated the repeatability of ASR as the intraclass correlation coefficient (ICC) (Lessells & Boag 1987), using only those species for which we had at least two ASR estimates from different populations. These analyses show a moderate repeatability of ASR, and that a significant part of ASR variation is interspecific (amphibians: $ICC = 0.559$, $F_{(22,96)} = 7.27$, $P < 0.001$, $n = 23$ species, n

= 120 records; reptiles: ICC = 0.524, $F_{(13,26)} = 4.11$, $P = 0.001$, $n = 14$ species, $n = 40$ records). For birds, our earlier analyses showed that nearly half (44%) of the ASR variation was interspecific, and that the direction of ASR (i.e. male- or female-biased) was highly conserved: in 44 species out of 55 (80%), the direction of ASR bias was the same for all repeated estimates (Székely *et al.* 2014b). For mammals, we did not have enough multiple ASR data within species to estimate repeatability.

In the comparative analyses we used the topology of Pyron & Wiens (Pyron & Wiens 2011) for amphibians, a composite phylogeny for reptiles (Gardner *et al.* 2008; Guillon *et al.* 2012; Pyron *et al.* 2013), topology of Jetz *et al.* (Jetz *et al.* 2012) for birds, and a composite phylogeny with the family-level relationships (Meredith *et al.* 2011) and the genus/species level relationships (Fritz, Bininda-Emonds & Purvis 2009) for mammals (Figure 7.1). For analyses across tetrapods, the branching topology between these four major clades was based on recent tetrapod phylogenies (Chiari *et al.* 2012; Amemiya *et al.* 2013, Figure 7.1). Since we did not have branch length information for these composite phylogenies, we ran the analyses using arbitrary gradual branch lengths according to Nee's method (Maddison & Maddison 2011). However, our results remained consistent when we repeated the analyses with other branch length assumptions (Pagel's method and unit branch lengths; see Table A/3.2 in Appendix A/3.).

To test the association between ASR bias (male- versus female-biased) and GSD (XY versus ZW) in phylogenetically corrected analyses, we used Pagel's discrete method (Pagel 1994) as implemented in *BayesTrait* (Pagel & Meade 2006). We used maximum likelihood methods to fit independent and dependent models for transitions in ASR bias and GSD states, and compared the fit of these two models by a likelihood ratio test (Pagel 1994). To test the ASR difference between XY and ZW species, we used PGLS models with maximum likelihood estimates of Pagel's lambda values (Pagel 1998) using the R package *caper* (Orme *et al.* 2013; R Core Team 2014). ASR was the response variable in all models, and genetic sex-determination system was fitted as the predictor (Table 7.1, Table A/3.1). The parameter estimate b shows the difference in ASR (proportion of males in the population) between ZW and XY species.

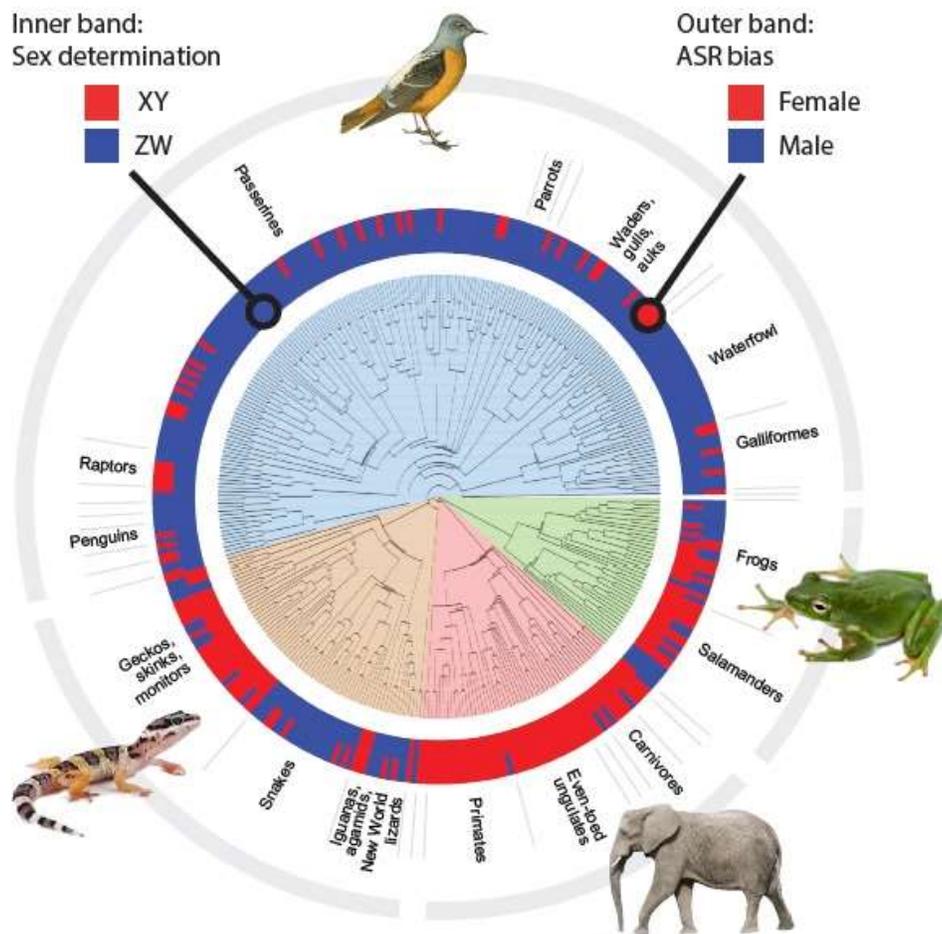


Figure 7.1. Phylogenetic distribution of adult sex ratio (ASR) and genetic sex-determination systems across tetrapods. Inner band shows the type of sex determination (red: XY, blue: ZW) and the outer band shows the ASR bias for each species included in the study (red: ≤ 0.5 , blue: > 0.5 proportion of males). Sample sizes: 39 species for amphibians, 67 species for reptiles, 187 species for birds and 51 species for mammals.

We also collected data on three additional ecological and behavioural variables to control for their known correlation with ASR in the PGLS model. First, we used body size (in mm) which was measured as snout to vent length for amphibians and squamates, and carapace length for the two turtle species, where possible from the same population for which ASR was reported. Head-body length was used for $n=36$ mammal species (Lislevand, Figuerola & Székely 2007). Since head-body length is not available for the vast majority of birds, we calculated this from the total body length by extracting bill and tail length ($n = 133$). Where we had sex-specific data, the mean of male and female head-body length was used as body size variable in the analyses. Second, we estimated sexual size dimorphism (SSD) as $\log_{10}(\text{male body size}) - \log_{10}(\text{female body size})$. For birds, we used body mass dimorphism (data available for $n = 181$

species, Lislevand, Figuerola & Székely 2007) due to the lack of sex-specific body length data. Third, we included breeding latitude (Iverson *et al.* 1993; Du *et al.* 2014) as the geographic coordinates of the ASR studies for amphibians and reptiles, taking absolute values to represent distance from the Equator in latitudinal degree. When the authors did not report latitude, we used Google Earth to estimate it on the basis of the description of the study site. For birds and mammals, we used the latitudinal midpoint of the breeding range of the species (n=182 and 44 species, for birds and mammals, respectively; sources: V. Remes, A. Liker, R. Freckleton and T. Székely unpublished data for birds, and the PanTHERIA database (Jones *et al.* 2009) for mammals, respectively). Mean values of these variables were used if multiple data of body size, latitude or size dimorphism per species were available. We could not find body size and latitude data for some species, thus sample sizes were reduced in multi-predictor models.

To test the robustness of the bivariate results, we added body size, breeding latitude and SSD as predictors in multi-predictor models to control for their potential confounding effects (Table 7.2). As in earlier ASR studies (Donald 2007; Székely *et al.* 2014b), the distribution of ASR values did not deviate significantly from normal in the four clades separately as well as in tetrapods as a whole (see additional analyses to validate our methods and results in Appendix A/3).

Table 7.1. The effect of the sex-determination system on the adult sex ratio.

Taxon	Number of species	Mean ASR				% species with male-biased ASR		
		XY	ZW	<i>t</i> -test [†]	PGLS [†]	XY	ZW	PDM [†]
Amphibians	39	0.51	0.61	**	**	42.9	90.9	*
Reptiles	67	0.45	0.57	***	***	24.2	76.5	*
Birds	187	--	0.55	--	--	--	76.5	--
Mammals	51	0.37	--	--	--	9.8	--	--
Tetrapods	344	0.43	0.55	***	***	22.3	77.2	***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, -- no data or not tested

[†]Detailed results of the statistical analyses are presented in Table A/3.1

Mean adult sex ratios (ASR, proportion of males in the population), *t*-tests and percentage of species with male-biased ASRs represent species-level statistics and analyses, while phylogenetic generalized least squares (PGLS) and Pagel's discrete method (PDM) were used for phylogenetically corrected analyses of the difference in ASR between XY and ZW species.

Other possible confounding factors include the lifespan of individuals and sex-specific dispersal distances, but according to our analyses on available data (presented in Appendix A/3 and A/3.1), lifespan is not related to ASR and we found no relationship between sex bias in dispersal and GSD or ASR. Additionally, data on lifespan and/or sex-specific dispersal are not available for most species in our ASR data set, we did not include these variables in the main analyses. Additional analyses as validation for the methods and results are presented in Appendix A/3.

Population genetic models

We developed population genetic models of the effects that deleterious mutation and sex-antagonistic selection might have on the ASR (Appendix A/3.2). The models assume that deleterious mutations are largely or entirely recessive, that they have multiplicative fitness effects across loci, that the loci are fully sex-linked and in linkage equilibrium, that mutation is not sex-biased, and that selection is strong relative to mutation and drift. Fitness effects of mutations in hemizygotes and homozygotes are assumed equal. Full details of the models are given in Appendix A/3.2. Here we summarize key results.

When deleterious alleles reach a mutation-selection balance, with X-Y sex determination the mean viability of males relative to females is

$$\bar{W}_m \approx \exp\{-3U_X - U_Y\},$$

where U_X and U_Y are the total rates of mutation to deleterious alleles across all loci on the X and Y chromosomes. With Z-W sex determination, the mean viability of females to males is

$$\bar{W}_f \approx \exp\{-3U_Z - U_W\},$$

where U_Z and U_W are the total rates of mutation to deleterious alleles across all loci on the Z and W chromosomes. Using very rough estimates for rates of deleterious mutations appropriate for human sex chromosomes, we estimate that mutation-selection balance might bias the ASR by a few percent. This degree of bias is substantially less than what is seen in our data. We emphasize that the conclusion could be quite different using other parameter values, or if the model was extended to include stochastic effects.

The second hypothesis to explain biased ASRs that we explored with models is sex-antagonistic selection, the situation in which alleles are selected differently in females and males. In Supplementary Material 2, we use numerical examples to show that under both XY and ZW sex determination, either a female-biased or male-biased ASR can result. Thus there does not seem to be a robust generalization about how sex-antagonistic selection will bias the ASR.

Results and discussion

Both the ASR and the sex-determination system are highly variable across tetrapods (Figure 7.1). We find that ASR and sex determination are correlated. Before controlling for phylogenetic effects, we find that ASRs are significantly more male-biased in species with ZW sex determination than in those with XY sex determination (Figure 7.2, Table 7.1, Table A/3.1). Similarly, the proportion of species with male-biased ASRs is greater among ZW than among XY species (Figure 7.1, Table 7.1). These differences are significant within amphibians, within reptiles, and across tetrapods as a whole (Table 7.1, Table A/3.1).

The pattern remains significant after controlling for phylogenetic effects. Both the mean of ASR across species (analyzed using PGLS) and the proportion of species with male-biased sex ratios (analyzed using PDM) are significantly different between XY and ZW systems within amphibians, within reptiles, and across tetrapods as a whole (Table 7.1, Table A/3.1). The effect is strong in clades with variation in sex determination: the type of genetic sex determination explains up to 24% of the interspecific variance in ASR among amphibians and 36% in reptiles (estimated using PGLS, Table A/3.2). The results remain significant when we treat three large clades with invariant sex-determination systems as a single datum each (snakes, ZW; birds, ZW; mammals, XY; Table A/3.1), when we make different assumptions about branch lengths in the phylogeny (Table A/3.2), and when we use arc-sine transformed ASR values transformed before PGLS analyses (amphibians: $b \pm SE = 0.10 \pm 0.03$, $t_{37} = 3.44$, $P = 0.001$, $n = 39$; reptiles: $b \pm SE = 0.12 \pm 0.02$, $t_{65} = 5.95$, $P < 0.001$, $n = 67$; tetrapods: $b \pm SE = 0.11 \pm 0.02$, $t_{342} = 5.24$, $P < 0.001$, $n = 344$).

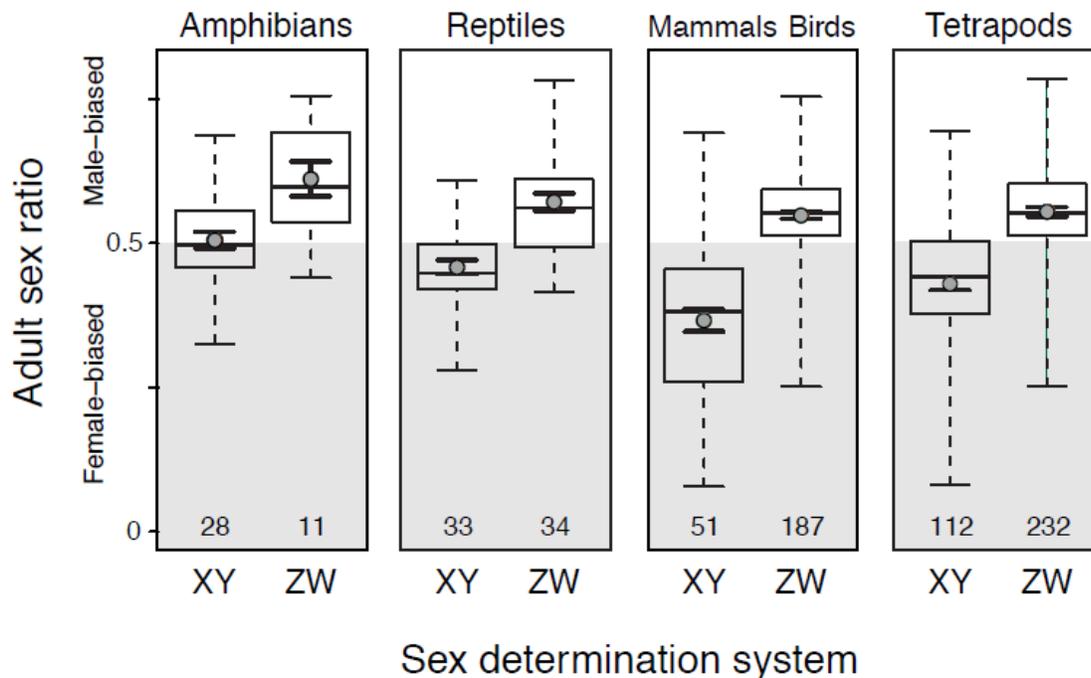


Figure 7.2. Variation in adult sex ratio as a function of the sex-determination system in amphibians, reptiles, mammals and birds, and tetrapods (all four clades combined). Adult sex ratio is the proportion of males in all adults. Central dots and solid whiskers are means \pm 1 SE, horizontal bars are medians, and boxes and dashed whiskers show the interquartile ranges and data ranges, respectively, based on species values. Numbers of species are at the bottom of each panel. See Table 7.1 and Table A/3.1 for statistical results and Figure A/3.1 for phylogenetically corrected graphs.

Body size and breeding latitude correlate with life-history traits in many organisms and these traits could affect ASR (Morrison & Hero 2003). Sexual size dimorphism is linked to differential sexual selection acting on males and females and thus influences sex-specific mortality, and has been suggested to drive the evolution of genetic sex-determination systems (Adkins-Regan & Reeve 2014). Nevertheless, we find that neither body size nor breeding latitude explains the ASR in phylogenetically controlled multi-predictor analyses (Table 7.2). Sexual size dimorphism is significantly associated with ASR in reptiles and across tetrapods as a whole, but the effect of the genetic sex-determination system remains significant when size dimorphism is included in the analysis (Table 7.2).

Table 7.2. The relationships between adult sex ratio, sex-determination system and other factors in phylogenetically corrected multi-predictor analyses.

	Amphibians (<i>n</i> = 39)			Reptiles (<i>n</i> = 67)			Tetrapods (<i>n</i> = 259)		
	<i>b</i> (\pm SE)	<i>t</i>	<i>P</i>	<i>b</i> (\pm SE)	<i>t</i>	<i>P</i>	<i>b</i> (\pm SE)	<i>t</i>	<i>P</i>
Sex-determination system	0.10 (\pm 0.03)	3.38	0.002	0.10 (\pm 0.02)	4.56	<0.001	0.10 (\pm 0.02)	5.23	<0.001
Body size	0. (\pm 0)	1.41	0.166	0 (\pm 0)	0.78	0.440	0 (\pm 0)	0.05	0.962
Breeding latitude	0 (\pm 0)	0.13	0.898	0 (\pm 0)	0.04	0.966	0 (\pm 0)	0.24	0.811
Sexual size dimorphism	-0.32 (\pm 0.34)	0.92	0.363	-0.31 (\pm 0.15)	2.17	0.034	-0.38 (\pm 0.07)	5.57	<0.001

Results of phylogenetic generalized least squares (PGLS). Separate models of ASR were constructed for amphibians, reptiles and tetrapods. For sex determination, *b* is the estimated difference in ASR between ZW and XY species.

Sex differences in dispersal may also result in biased ASRs. However, dispersal is unlikely to explain the relationship between ASR and sex-determination systems. First, male-biased dispersal is typical in reptiles regardless of sex-determination system (Qi *et al.* 2013). Second, there is no relationship between ASR and sex bias in dispersal distance in birds (Appendix A/3.1). Finally, the relationship between sex determination and ASR remain significant when the influence of sex-biased dispersal is controlled for in multi-predictor models in tetrapods (Appendix A/3.1).

The sex-determination system may affect the ASR in the directions seen in the data in a number of ways. First, sexual selection can fix mutations that increase male mating success and decrease male survival. These will accumulate on Y but not W chromosomes, and will accumulate more readily on X than on Z chromosomes if they tend to be recessive. Second, biased ASRs could result from recessive mutations at loci carried on the X (or Z) but absent from the Y (or W) chromosome since they are not masked in the heterogametic sex (the “unguarded sex chromosome” hypothesis) (Haldane 1922; Trivers 1972; Donald 2007), and from deleterious mutations carried on the Y (or W) but not on the X (or Z). At loci carried on both sex chromosomes, alleles on the Y (or W) can show partial degeneration (Bachtrog 2006). Population genetic models suggest that deleterious mutation pressure alone may not be adequate to explain ASR biases as large as those observed (Appendix A/3.2), but the models do not include factors that could be important, notably degeneration of Y and W chromosomes by genetic drift (Bachtrog 2006). A third hypothesis is imperfect dosage compensation, which may be deleterious to the heterogametic sex (Mank 2013). Fourth, distorted sex ratios can result from meiotic drive acting on sex chromosomes (Jaenike 2001). Drive more often produces female-biased sex ratios in XY

systems at birth (Werren & Beukeboom 1998). There is little data on drive in ZW systems, but if it operates in a symmetrical fashion then we expect it to cause male-biased sex ratios. Fifth, Y and W chromosomes might degenerate during the lifespan, for example by telomere shortening or loss of epigenetic marks, more rapidly than X and Z chromosomes. A final possibility is that sex-antagonistic selection acting on sex-linked loci could lead to biased sex ratios, but unlike the preceding hypotheses there does not seem to be a robust prediction about the direction of the ASR bias it will produce (Appendix A/3.2).

The limited data available do not provide clear support for any of these hypotheses, although critical tests are lacking. For instance, the meiotic drive process predicts biased sex ratios at birth. Although a recent comparative analysis in birds suggests that sex ratios at birth are unrelated to biased ASRs (Székely *et al.* 2014a), offspring sex ratios have not been compared between different sex-determination systems. Additional insight might come from study of dioecious plants with biased sex ratios (Field, Pickup & Barrett 2013), but their skewed ASR could result from selection on the gametophytic stage that is absent from animals (Hough *et al.* 2013). Evolutionary feedbacks from the ASR to the sex-determination system are also possible: for example, the ASR could influence sexual size dimorphism and sexual conflict, which in turn could trigger transitions in sex determination (Roberts, Ser & Kocher 2009; van Doorn & Kirkpatrick 2010; Adkins-Regan & Reeve 2014).

In conclusion, we demonstrate strong and phylogenetically robust associations between genetic sex-determination systems and a demographic property of populations, ASR. Although the mechanisms that drive this association need further theoretical and empirical analyses, the observed pattern is biologically important for two reasons. First, changes in sex-determination systems are expected to have knock-on effects on social behaviour. Theory suggests that ASR affects violence, pair bonds, infidelity and parental care (Kokko & Jennions 2008), and field-based studies support these predictions (Liker *et al.* 2013, 2014; Schacht *et al.* 2014). For instance, female-biased ASRs co-occur with polygyny and female care, whereas male-biased ASRs tend to co-occur with polyandry and male care in birds (Liker *et al.* 2013). Second, sex-determination systems may have important demographic consequences through skewed birth sex ratios and sex-biased survival. Such biases may not only impact upon the productivity and growth of populations, but also their genetic composition and viability. Further theoretical, experimental and comparative studies are clearly needed to understand the linkages between sex determination, demography and social behaviour.

8. General conclusions

The results of this thesis about effects of extreme weather events on reproductive success and offspring quality further support the studies highlighting that general patterns are rare about effects of weather extremities (Palmer *et al.* 2017), and general predictions about species' response to the projected future weather conditions are hard to make without detailed research on several populations examined under different environmental conditions. Responses to extremities can differ even between closely related taxa or between species living in the same environment but with different evolutionary origins (Kaiser, Merckx & Van Dyck 2016; Marrot, Garant & Charmantier 2017; Palmer *et al.* 2017). Moreover, studies often focus on population dynamics or community structures when they explore the effects of climate change (e.g. Jiguet, Brotons & Devictor 2011; Şekercioğlu, Primack & Wormworth 2012; Krause *et al.* 2017) and habitat urbanization (e.g. Chace & Walsh 2006; Schütz & Schulze 2015; Morelli *et al.* 2016), but changes in whole community structures is often a result of several mechanisms induced by weather conditions and/or urbanisation. Investigating the variation in population-level responses and adaptations to weather and habitat variability would help us to understand the mechanisms how these human-induced environmental changes act, and to realize potential threats and mitigation possibilities.

Investigating two successfully urbanized passerine species, we found that several components of reproductive success were affected by weather, like hatching success or offspring size. Populations of a species living in different habitat can also differ in their reaction to extreme weather (as we showed it in chapter 4). Effects of extremities can depend on which reproductive stage is investigated, as higher number of extreme hot days were advantageous during incubation but were harmful during chick rearing on the growth of the nestlings (chapter 3). Despite the facts that temperature was higher and reproductive success was lower in cities, we surprisingly found that breeding performance of great tits was more sensitive to extreme heat in natural forest habitat than in urban habitat type (chapter 4). Both fledging success and average body mass of broods showed more rapid decrease with increasing number of hot days in forests than in urban populations. Maintaining smaller body size in warmer urban environments can be an adaptive strategy (Merckx *et al.* 2018), and our results in a passerine bird corroborate it. Moreover, investigating individuals of 52 bird species over a 40-year period, researchers found that increasing annual summer temperature predicts consistent reductions in both body mass and tarsus length of nearly all species (Weeks *et al.* 2019), suggesting that warming-induced body size reduction can be a general response to climate change.

Our studies also showed that weather and habitat urbanisation did not change the sex allocation and skew the offspring sex ratios in house sparrows and great tits (chapters 3 & 5), as we found 1:1 offspring sex ratio in all investigated populations. We also found some evidence in both species that this might be because male and female nestlings may not differ considerably in their sensitivity to the early-development environment. It seems that highly skewed juvenile sex ratios are not a threat in urban populations at least in our study species, but results about the offspring sex ratios in urban populations are miscellaneous (Rejt *et al.* 2005; Bonderud *et al.* 2017). Nevertheless, parents may allocate differently into alternative reproductive strategies according to habitat urbanisation. Namely, we found that extra-pair fertilizations in great tits occurred more frequently in urban than in natural habitats (chapter 6), and the direction of this pattern was similar in all other studies that investigated this phenomenon (Moore *et al.* 2012; Rodriguez-Martínez *et al.* 2014; Rosenfield *et al.* 2015). The increased promiscuity in urban habitats may help to maintain genetic diversity, as in urban populations inbreeding can be greater (Shaw, Chamberlain & Evans 2008; Vangestel *et al.* 2011). Further molecular studies investigating the genetic parentage of urban populations would be important to find whether it is a general pattern with urbanisation, and studies needed also to explore the drivers of higher promiscuity in urban populations.

Investigating adult sex ratios beside the offspring sex ratio can be a new interest of sex allocation studies as it can be an important aspect of mating system and parental care evolution (Kokko & Jennions 2008; Székely *et al.* 2014b; Schacht *et al.* 2017). Frequency-dependent selection of ASR is expected, as the rarer sex in the population has more opportunities to choose a mate and/ or abandon the partner than the more common sex, so population ASRs can influence the reproductive decisions of individuals. Phylogenetic analyses of birds are consistent with these propositions (Liker *et al.* 2013, 2014). ASR is usually male-biased in birds (Donald 2007), and this is true for both house sparrows (Anderson 2006; Liker *et al.* 2014) and great tits (Liker *et al.* 2014). However, we found no deviations from 1:1 sex ratio neither at conception nor at fledging. Our result is consistent with results for most birds that have been studied to date (Donald 2007; Székely *et al.* 2014a). These findings suggest that the sex ratio becomes skewed towards males after (not before) fledging in these species. Paralell with this, a comparative study on birds has found that sex-biased mortality of adults was the most likely predictor of adult sex ratio, whereas sex ratio at hatching was not related to sex ratio in adulthood (Székely *et al.* 2014a). Further studies are needed to investigate the transitions in sex ratios from conception till adulthood in relation to urbanization and climate change.

Despite the long tradition of investigating sex ratios, there are still a couple of questions about how the variability in population sex ratios can emerge. Our phylogenetic comparative study found that adult

sex ratio differences among tetrapod species can have genetic basis via sex chromosome systems (chapter 7). We showed that the heterogametic sex is underrepresented in the adult populations as suggested by the unguarded sex chromosome hypothesis (Haldane 1922; Trivers 1972; Donald 2007). Although the mechanisms causing this relationship remain untested, this result is an important step for studying evolution of genome (Kirkpatrick 2017) and variation in sex determination systems (Sabath *et al.* 2016). Adult sex ratio can be an important link between evolution of sex determination systems and population dynamics, demography and behaviour, but further research is needed to explore the causal relationships. Theoretical research indicates that the type of sex-chromosome system not only can influence the adult sex ratio but may also determine what effects climate change would have on the species' demography and genomic evolution (Bókony *et al.* 2017). Anyway, since all birds have the ZW/ZZ sex chromosome system, and birds are over-represented in ecological research, it will be important to expand and deepen our research efforts on other taxa with diverse sex-determination modes to understand and predict the effects of anthropogenic environmental change.

My research demonstrated some aspects of how populations can react to human-induced environmental change. The results contributed to understand the responses of wild animals to processes what are entrained with climate change and urbanisationhuman society urgently have to deal with. Additionally, I would like to highlight the importance of studying population-level responses of both demographical and reproductive traits related to climate change and habitat alterations to deepen our knowledge and find solutions. As Jane Goodall said "*You cannot get through a single day without having an impact on the world around you. What you do makes a difference, and you have to decide what kind of difference you want to make.*"

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10. Thesis points

Effects of extreme weather on the reproductive success of house sparrows and great tits

1. I showed that weather can have different effects on different components of breeding success in cavity-nesting passerine species. In house sparrows, I found that average and extreme temperatures had opposite effects. Average warm weather was advantageous for reaching greater fledgling size, but offspring size (body mass and tarsus length) decreased with increasing number of hot days, which may decrease the future survival of offspring. On the other hand, unusual heat increased hatching success, which may occur because high temperature is advantageous for embryonic development.
2. Investigating two urban and two forest populations of great tits, I found evident urban heat island effect, as birds in our urban sites experienced on average 1.53 °C and maximum 6.3 °C higher temperatures than in our forest study sites. The number of hot days in urban sites ranged between 0 and 13 whereas it was between 0 and 5 in forest sites. I found that the average body mass of a brood decreased with increasing number of hot days in the two forests and one of the urban populations, while the relationship between average body mass and number of hot days was positive at the hottest urban site. The average tarsus length of broods was not influenced by the number of hot days. This suggests that extreme heat may have negative effect through the increased metabolic energy requirement and/or dehydration of the nestlings on cooler sites, rather than through food availability. In the hottest urban population, the positive effect of hot days suggests heat adaptation.

Offspring sex ratio and extra-pair offspring in different habitat types

3. I found that offspring sex ratio did not deviate from 1:1 either in house sparrows nor in urban and forest populations of great tits. Sex ratio at fledging was not affected by weather conditions in house sparrows, and it did not differ between forest and urban habitats in great tits. According to these results, average and extreme weather conditions and habitat urbanisation may not affect sex allocation in these species. These results suggest that, under the investigated environmental conditions, passerines may not benefit from biasing their offspring sex ratio and sensitivity of male and female offspring may not differ considerably.
4. Investigating great tit families in urban and forest habitat types, I found consistently higher frequency of broods containing extra-pair offspring in cities than in forests. This result suggests that the altered ecological conditions of urban habitats affect the reproductive decisions of individual birds. Urban individuals may have more spatiotemporal opportunities than forest birds to meet other partners because of higher breeding density or altered breeding synchrony. Additionally, urban females may

have more motivation to find extra-pair males because of altered quality signals (e.g. song, plumage) in cities caused by pollution. The increased frequency of extra-pair fertilizations may also impact on the genetic diversity of urban-dwelling populations.

Relationship between type of genetic sex determination and adult sex ratio

5. In a comparative research on tetrapod (amphibian, reptile, bird, mammal) species, I showed that the direction of adult sex ratio bias is related to the type of genetic sex-determination system. Species with XX/XY sex-chromosome system (mammals, some reptiles and some amphibians) more frequently have female-biased adult sex ratios while species with ZZ/ZW sex-chromosome system (birds, some reptiles and some amphibians) are more likely to have male-biased adult sex ratios. This result agrees with the *unguarded sex chromosomes hypothesis*, but it may also be explained by several possible mechanisms. For example, meiotic drive acting on sex chromosomes or sex antagonistic selection on sex-linked loci both can cause the observed bias in adult sex ratio.

11. Tézispontok

Szélsőséges időjárás hatásai házi verebek és széncinegék szaporodási sikerére

1. Kimutattam, hogy az időjárás hatása változatos az odúköltő énekesmadárfajok szaporodási sikerének több komponensére. Házi verebeknél az átlagos és extrém hőmérsékletek ellentétes hatását találtam a fiókák méreteire, ugyanis az átlagosan melegebb hőmérséklet kedvezett a fiókák fejlődésének, azonban a sok hőségnap a fiókaméretet (testtömeg és csüd hossz) csökkenésével járt együtt, ami csökkentheti a fiókák túlélési esélyeit. Ugyanakkor az extrém meleg növelte a tojások kelési sikerét, ami valószínűleg a magas hőmérsékletnek az embriófejlődésre gyakorolt kedvező hatásának köszönhető.
2. Két városi és két erdei széncinege populációt vizsgálva kimutatható városi hősziget hatást találtam: a városi élőhelyen átlagosan 1.53 °C-kal, maximum akár 6.3°C-kal tapasztaltak melegebb környezetet a városban élő madarak az erdei fajtársaikhoz képest. Az extrém meleg napok száma 0-13 között változott a városi, míg 0-5 között az erdei helyszíneken. A szaporodási sikerrel összefüggésben azt találtam, hogy az extrém meleg napok számának növekedésével a széncinege fiókák testtömege csökkent a két erdei és az egyik városi élőhelyen, míg a legmelegebb városi helyszínen a fiókatömeg pozitív összefüggést mutatott az extrém meleg napok számával. A széncinege fiókák csüd hosszát az extrém meleg napok száma nem befolyásolta. Ez arra enged következtetni, hogy az extrém meleg nem a táplálék-elérhetőségen keresztül, hanem inkább a hőstressz miatti megnövekedett metabolizmus energiaigénye és/vagy a dehidrátság miatt hathat negatívan a fiókatömege a hűvösebb élőhelyeken. A meleg városi körülmények között élő populációban (Balatonfüreden) pedig hőszéles adaptációra utal az, hogy a magas hőmérsékletek egyedül itt voltak kedvezőek a fiókanevelési sikernek.

Utódok ivararánya és extra-pár utódok különböző élőhelytípusokban

3. Az utód-ivararány szuburbán házi veréb, valamint városi és erdei széncinege fészekaljok vizsgálata alapján nem mutatott eltolódást egyik élőhelyen sem az 1:1 aránytól, illetve nem különbözött az erdei és városi élőhelyek között széncinegékénél. Eredményem szerint sem az átlagos vagy extrém időjárási hatások, sem az élőhely-urbanizáció nem befolyásolták az ivari allokációt, ami arra enged következtetni, hogy az általunk vizsgált körülmények között az énekesmadaraknak nem éri meg kiegyenlítetlen ivararányt létrehozni, illetve a hím és tojó utódok érzékenysége nem tér el ezen hatások vizsgált tartományaiban.

4. Városi és erdei élőhelyen élő széncinege populációk összesen 86 családját és 1010 egyedét genotipizálva azt találtam, hogy az extra-pár utódok előfordulási gyakorisága magasabb városban, mint erdőben. Ez az eredmény azt sugallja, hogy az urbanizáció által megváltozott ökológiai és környezeti viszonyok hatással vannak az egyedek szaporodási döntéseire. A városi egyedeknek a magasabb költési egyedsűrűség és az eltérő költési szinkronitás miatt több lehetőségük lehet térben és időben is más partnerekkel találkozni, illetve a városokra jellemző fény- vagy zajszennyezés miatti megváltozott minőségjelző szignálok (pl. ének, tollazat) miatt a városi tojóknak nagyobb lehet a motivációja a jó minőségű partnerek keresésére, mint az erdei környezetben élő egyedeknek. A gyakoribb városi extra-pár fertilizációk hatással lehetnek a városi madárpopulációk genetikai diverzitására is.

Az ivari kromoszóma-rendszer típusa és a felnőttkori ivararány változatossága

5. A tetrapoda klád (kétéltű, hüllő, madár, emlős) fajait vizsgálva kimutattam, hogy a felnőttkori ivararány eltolódásának az iránya összefüggésben van a genetikai ivardeterminációs rendszerrel. XY/XX kromoszóma rendszerű fajoknál (emlősök, egyes hüllők és egyes kétéltűek) a nőstény túlsúlyos ivararány jellemzőbb, míg ZZ/ZW kromoszóma rendszerrel rendelkező fajoknál (madarak, egyes hüllők és egyes kétéltűek) pedig a hím túlsúlyos felnőttkori ivararány jellemző. Ez az eredmény összhangban van a „védtelen ivari kromoszóma hipotézis”-sel, azonban az ivari kromoszóma rendszerek több más mechanizmuson keresztül is befolyásolhatják a felnőttkori ivararányt a kapott mintázat, azaz a heterogametikus ivar relatív hiánya irányában. Például az ivari kromoszómákon ható meiotikus drive és ivari lókuszokon kialakuló ivari-antagonista szelekció is okozhatja a felnőttkori ivararányok eltolódását.

12. Publications

Papers included in the dissertation:

1. **Pipoly, I.**, Szabó, K., Bókony, V., Preiszner, B., Seress, G., Vincze, E., Schroeder, J., Liker, A. (2019): Higher frequency of extra-pair offspring in urban than forest broods of great tits (*Parus major*), *Frontiers in Ecology and Evolution*, 7:226
2. **Pipoly, I.**, Bókony, V., Kirkpatrick, M., Donald, P., Székely, T., Liker, A. (2015): The genetic sex-determination system predicts adult sex ratios in tetrapods, *Nature*, 527(7576), 91-94 [IF: 38.14]
3. **Pipoly, I.**, Bókony, V., Seress, G., Szabó, K., Liker, A. (2013): Effects of extreme weather on reproductive success in a temperate-breeding songbird, *PLoS ONE*, 8 (11), 1-11, [IF: 3.53]

Papers not included in the dissertation:

1. Vincze, E., **Pipoly, I.**, Seress, G., Preiszner, B., Papp, S., Németh, B., Liker, A., Bókony, V. (2019): Great tits take greater risk toward humans and sparrowhawks in urban habitats than in forests, *Ethology*, 125(10), 686-701
2. Bókony, V., Milne, G., **Pipoly, I.**, Székely, T., Liker, A. (2019): Sex ratios and bimaturism differ between temperature-dependent and genetic sex-determination systems in reptiles, *BMC Evolutionary Biology* 19:57 DOI:<https://doi.org/10.1186/s12862-019-1386-3>
3. Seress, G., Hammer, T., Bókony, V., Vincze, E., Preiszner, B., **Pipoly, I.**, Sinkovics, Cs., Evans, K. L., Liker, A. (2018): Impact of urbanization on abundance and phenology of caterpillars and consequences for breeding in an insectivorous bird, *Ecological Applications* 28 : 5 pp. 1143-1156
4. Seress, G., Vincze, E., **Pipoly, I.**, Hammer, T., Papp, S., Preiszner, B., Bókony, V. Liker, A. (2017): Effects of capture on the behavior and breeding success of urban and forest Great Tits, *Journal of Field Ornithology*, DOI: 10.1111/jfo.12205 [IF:1.51]
5. Bókony, V., **Pipoly, I.**, Szabó, K., Preiszner, B., Vincze, E., Papp, S., Seress, G., Hammer, T., Liker, A. (2017): Innovative females are more promiscuous in great tits (*Parus major*), *Behavioural Ecology*, 28(2), 579-588 [IF: 3.03]
6. Preiszner, B., Papp, S., **Pipoly, I.**, Seress, G., Vincze, E., Liker, A., Bókony, V. (2017): Problem-solving performance and reproductive success of great tits in urban and forest habitats, *Animal Cognition* 20 (1), 53-63 [IF: 2.16]

7. **Pipoly Ivett**, Preiszner Bálint, Seress Gábor, Vincze Ernő, Liker András (2014): Éghajlatváltozás erdőn, mezőn: a vadon élő állatok kutatásának tanulságai, *Iskolakultúra*, 11-12, 103-114 o.
8. Seress, G., Bókony, V., **Pipoly, I.**, Szép, T., Nagy, K., Liker, A. (2012): Urbanization, nestling growth and reproductive success in a moderately declining house sparrow population, *Journal of Avian Biology*, 43, 1-12; [IF: 2.28]
9. **Pipoly Ivett**, Bókony Veronika, Liker András (2011): Hogyan befolyásolja az időjárás a házi verebek (*Passer domesticus*) fiókaetetési viselkedését? *Állattani Közlemények*, 96 (1-2): 97-111

Oral conference presentations

1. **Pipoly, I.**, Bókony, V., Liker, A. (2009): Hogyan befolyásolják a klimatikus tényezők a házi verebek (*Passer domesticus*) fiókaetetési viselkedését?, Tudományos Diákköri Konferencia, SZIE ÁOTK (Budapest) – magyar nyelvű előadás, 2. helyezés
2. **Pipoly, I.**, Bókony, V., Liker, A. (2010): Hogyan befolyásolják a klimatikus tényezők a házi verebek (*Passer domesticus*) fiókaetetési viselkedését?, XII. Országos Felsőoktatási Környezettudományi Diákkonferencia (Sopron) - magyar nyelvű előadás, különdíj
3. Seress, G., Bókony, V., **Pipoly, I.**, Liker, A. (2010): Differences in breeding success and nestling development between rural and urban house sparrows. International Student Conference of Carpathian-Basin (TUDOC), (Gödöllő) - angol nyelvű előadás
4. **Pipoly, I.**, Bókony, V., Kis, J., Szabó, K., Liker, A. (2011): Hogyan befolyásolja az időjárás a házi verebek (*Passer domesticus*) fiókaetetési viselkedését?, XXX. Országos Tudományos Diákköri Konferencia, Biológia Szekció, Viselkedésokológia Tagozat (Budapest) - magyar nyelvű előadás, 2. helyezés
5. **Pipoly, I.**, Bókony, V., Szabó, K., Liker, A. (2012): Extrém időjárási tényezők hatása a házi verebek (*Passer domesticus*, L.) szaporodási sikerére, XIV. Magyar Etológiai Társaság Kongresszus (Kolozsvár, Románia) - magyar nyelvű előadás
6. **Pipoly, I.**, Bókony, V., Seress, G., Szabó, K., Liker, A. (2013): Extrém időjárási tényezők hatása a házi veréb (*Passer domesticus* L.) szaporodási sikerére, Magyar Biológiai Társaság Állattani Szakosztály előadóiülése (Budapest) - magyar nyelvű előadás
7. **Pipoly, I.**, Bókony, V., Liker, A., Székely, T. (2013): Az ivari kromoszómák és a felnőttkori ivararány kapcsolatának filogenetikai vizsgálata hüllőknél, XV. Magyar Etológiai Társaság Kongresszus (Budapest) - magyar nyelvű előadás

8. Bókony, V., Hammer, T., Papp, S., **Pipoly, I.**, Preiszner, B., Seress, G., Sinkovics, B.C., Vincze, E., Liker, A. (2014): Miért csökkenti az urbanizáció a széncinegék szaporodási sikerét? V. Kvantitatív Ökológiai Szimpózium (Tihany) - magyar nyelvű előadás
9. **Pipoly, I.**, Bókony, V., Székely, T., Liker, A. (2014): Adult sex ratios are biased towards the homogametic sex in amphibians and reptiles, "7th European Conference on Behavioural Biology", (Prága, Cseh Köztársaság) – angol nyelvű előadás
10. **Pipoly, I.**, Hammer, T., Papp S., Preiszner B., Seress G., Szabó K., Vincze, E., Bókony, V., Liker, A. (2014): Extra-pár utódok gyakorisága erdei és városi széncinege (*Parus major*) populációkban, XVI. Magyar Etológiai Társaság Kongresszus (Tihany) – magyar nyelvű előadás
11. Liker, A., **Pipoly, I.**, Bókony, V., Székely, T. (2014). Hogyan befolyásolja az ivararány az ivari szelekciót?, XVI. Magyar Etológiai Társaság Kongresszus (Tihany) – magyar nyelvű előadás
12. Vincze, E., **Pipoly, I.**, Hammer, T., Papp, S., Preiszner, B., Seress, G., Németh, B., Bókony, V., Liker, A. (2014). Emberrel és ragadozókkal szembeni viselkedési válaszok városi és vidéki énekesmadaroknál, XVI. Magyar Etológiai Társaság Kongresszus (Tihany) – magyar nyelvű előadás
13. Papp, S., Hammer, T., Vincze, E., Preiszner, B., **Pipoly, I.**, Seress, G., Liker, A., Bókony, V. (2014). Az urbanizáció hatása a neofóbiára és a problémamegoldó képességre házi és mezei verebeknél, XVI. Magyar Etológiai Társaság Kongresszus (Tihany) – magyar nyelvű előadás
14. Seress, G., Papp, S., **Pipoly, I.**, Preiszner, B., Vincze, E., Liker, A., Bókony, V. (2014). A méret a lényeg: az élőhely-urbanizáció hatása két verébfaj fiókaetelési viselkedésére, XVI. Magyar Etológiai Társaság Kongresszus (Tihany) – magyar nyelvű előadás
15. Hammer, T., Sinkovics, Cs., Bókony, V., Papp, S., **Pipoly, I.**, Somogyi, R., Evans, K., Liker, A. (2015): Lepkehernyók mennyiségének és időbeli változásának vizsgálata erdei és városi területeken, X. Magyar Ökológus Konferencia (Veszprém) – magyar nyelvű előadás
16. Németh, B., Vincze, E., **Pipoly, I.**, Papp, S., Preiszner, B., Seress, G., Hammer, T., Liker, A., Bókony, V. (2015): Széncinegék (*Parus major*) ragadozóval szembeni viselkedése az élőhelyurbanizáció függvényében, X. Magyar Ökológus Konferencia (Veszprém) – magyar nyelvű előadás
17. Papp, S., Vejnovic, A., Hammer, T., Vincze, E., Preiszner, B., **Pipoly, I.**, Seress, G., Bókony, V., Liker, A., (2015): Városi és erdei széncinegék (*Parus major*) kockázatvállaló viselkedésének vizsgálata a fészkelés alatt, X. Magyar Ökológus Konferencia (Veszprém) – magyar nyelvű előadás
18. Seress, G., Hammer, T., Papp, S., **Pipoly, I.**, Preiszner, B., Vincze, E., Liker, A. (2015): Hogyan befolyásolja az odúcsapdás befogás a széncinegék utódgondozó magatartását?, XVII. Magyar Etológiai Társaság Kongresszus (Dobogókő) – magyar nyelvű előadás

19. Szabó, K., **Pipoly, I.**, Preiszner, B., Hammer, T., Papp, S., Seress, G., Vincze, E., Liker, A., Bókony, V. (2015): Promiszkuitás és problémamegoldási siker széncinegéknél (*Parus major*), XVII. Magyar Etológiai Társaság Kongresszus (Dobogókő) – magyar nyelvű előadás
20. Preiszner, B., Papp, S., **Pipoly, I.**, Seress, G., Vincze, E., Liker, A., Bókony, V. (2015): Urbanization, problem-solving and reproductive success in Great Tits *Parus major*, 10th Conference of the European Ornithologist's Union (Badajoz, Spanyolország) – angol nyelvű előadás
21. Sinkovics, Cs., Seress, G., **Pipoly, I.**, Hammer, T., Vincze, E., Papp, S., Preiszner, B., Liker, A. (2016): Ivari és élőhelyi különbségek széncinegék (*Parus major*) utódgondozó viselkedésében, XVIII. Magyar Etológiai Társaság Kongresszus (Debrecen) – magyar nyelvű előadás
22. **Pipoly, I.**, Bókony, V., Donald, P. F., Hammer, T., Kirkpatrick, M., Papp, S., Preiszner, B., Schroeder, J., Seress, G., Szabó, K., Székely, T., Vincze, E., Liker, A. (2016): Környezeti tényezők hatása az ivararányra és szaporodási sikerre, XVIII. Magyar Etológiai Társaság Kongresszus (Debrecen) – magyar nyelvű plenáris előadás az “Év ifjú viselkedéskutatója 2016” díj nyerteseként
23. Vincze, E., Bókony, V., **Pipoly, I.**, Seress, G., Preiszner, B., Papp, S., Németh, B., Liker, A. (2017): Széncinegék emberrel és karvallyal szembeni viselkedése városban és vidéken: általános vagy ragadozó-specifikus kockázatvállalási viselkedés? XIX. Magyar Etológiai Társaság Kongresszus (Dobogókő) – magyar nyelvű előadás
24. **Pipoly I.:** A szaporodási sikert befolyásoló környezeti tényezők, Intézményi ÚNKP Konferencia, Pannon Egyetem Mérnöki Kar, Veszprém, 2018. 06. 01. – magyar nyelvű előadás
25. Czikkelyné Ágh, N., **Pipoly, I.**, Bókony, V., Vincze, E., Seress, G., Szabó, K., Liker, A., 2018. Környezeti tényezők és a szülők testméreteinek hatása a fészekalj ivararányára széncinegéknél. XIX. Kolozsvári Biológus Napok, Kolozsvár, Románia - magyar nyelvű előadás
26. Vincze, E., Bókony, V., **Pipoly, I.**, Seress, G., Preiszner, B., Papp, S., Németh, B., Liker, A., 2018. Széncinegék emberrel és karvallyal szembeni viselkedése városban és erdőben: általános vagy veszély-specifikus kockázatvállalási viselkedés? XIX. Kolozsvári Biológus Napok, Kolozsvár, Románia - magyar nyelvű előadás
27. Bukor, B., Bókony, V., Hammer, T., Papp, S., **Pipoly, I.**, Preiszner, B., Seress, G., Sinkovics, Cs., Vincze, E., Liker, A., 2018: Éven belüli és évek közötti újrafészkelések gyakorisága, erdei és városi széncinege populációkban. XIX. Kolozsvári Biológus Napok, Kolozsvár, Románia - magyar nyelvű előadás
28. Seress, G., Hammer, T., Bókony, V., Vincze, E., Preiszner, B., **Pipoly, I.**, Sinkovics, Cs., Evans, K.L., Liker, A., 2017. Impact of urbanization on abundance and phenology of caterpillars and consequences

- for breeding in an insectivorous bird. Eighth International Hole-Nesting Birds Conference, Trondheim, Norvégia - angol nyelvű előadás
29. Czikkelyné Ágh N., Rónai Zs., Molnár K., Bókony V., Vincze E., Preiszner B., Seress G., **Pipoly I.**, Liker A. (2018): DRD4 gén polimorfizmus összefüggése széncinegék neofóbiájával és problémamegoldó viselkedésével – egy elővizsgálat eredményei, XX. Magyar Etológiai Társaság Kongresszus (Kolozsvár) – magyar nyelvű előadás
30. Vincze E., Seress G., **Pipoly I.**, Sinkovics Cs., Sándor K., Liker A. (2018): Élőhelyi különbségek a széncinegék fészekvédő viselkedésében, XX. Magyar Etológiai Társaság Kongresszus (Kolozsvár) – magyar nyelvű előadás
31. Vincze E., Bókony V., **Pipoly I.**, Seress G., Preiszner B., Papp S., Németh B., Liker A. (2018): Széncinegék emberrel és karvallyal szembeni viselkedése városban és erdőben: általános vagy veszély-specifikus kockázatvállalási viselkedés?, 19. Kolozsvári Biológus Napok (Kolozsvár) – magyar nyelvű előadás
32. Vincze E., Bókony V., **Pipoly I.**, Seress G., Preiszner B., Papp S., Németh B., Liker A. (2018): Effects of urbanization on risk taking by great tits: general syndrome or threat-specific responses?, ISBE 2018 – 17th International Behavioral Ecology Congress (Minneapolis, Minnesota, USA) – angol nyelvű előadás
33. Sinkovics Cs., Seress G., **Pipoly I.**, Vincze E., Liker A. (2018): Urbanizáció hatása széncinegék (*Parus major*) fiókaetelési viselkedésére, 1. Urbanizációs Ökológia Konferencia (Veszprém) – magyar nyelvű előadás
34. Sinkovics Cs., Seress G., **Pipoly I.**, Vincze E., Liker A. (2018): Urbanizáció hatása széncinegék (*Parus major*) fiókaetelési viselkedésére, XX. Magyar Etológiai Társaság Kongresszus (Kolozsvár) – magyar nyelvű előadás
35. Seress G., Hammer T., Bókony V., Vincze E., Preiszner B., **Pipoly I.**, Sinkovics Cs., Evans, K:E., Liker A. (2018): A lombfakadás, a hernyók szezonális és tömegessége, és a széncinegék szaporodási sikere közötti összefüggések és eltérések városi és természetes élőhelyeken, 1. Urbanizációs Ökológia Konferencia (Veszprém) – magyar nyelvű előadás
36. Bukor B., Bókony V., Hammer T., Papp S., **Pipoly I.**, Preiszner B., Seress G., Sinkovics Cs., Vincze E., Liker A. (2018): Éven belüli és évek közötti újrafészkelések gyakorisága, erdei és városi széncinege populációkban, 19. Kolozsvári Biológus Napok (Kolozsvár) – magyar nyelvű előadás

37. Bukor B., Czikkelyné Ágh N., **Pipoly I.**, Sándor K., Seress G., Sinkovics Cs., Vincze E. Liker A. (2018): Széncinegék túlélésének vizsgálata különböző típusú fészkelő helyeken, 1. Urbanizációs Ökológia Konferencia (Veszprém) – magyar nyelvű előadás
38. Bukor B., Czikkelyné Ágh N., **Pipoly I.**, Sándor K., Seress G., Sinkovics Cs., Vincze E. Liker A. (2018): Széncinegék túlélésének vizsgálata különböző típusú fészkelő helyeken, XX. Magyar Etológiai Társaság Kongresszus (Kolozsvár) – magyar nyelvű előadás
39. Liker A., Bókony V., Papp S., **Pipoly I.**, Preiszner B., Seress G., Szabó K., Vincze E. (2018): Élőhelyi különbségek a madarak problémamegoldási sikerében, "Botanikai és Zoológiai Kutatások Épített Környezetünkben" c. MTA előadóiülés (Budapest) – magyar nyelvű előadás
40. Liker, A., Bókony, V., **Pipoly I.**, Lemaître, J-F., Gaillard, J-M., Székely T., Freckleton, R.P. (2018): Sexual size dimorphism is associated with adult sex ratios in birds and other tetrapods, 27th International Ornithological Congress (Vancouver, Canada) – angol nyelvű előadás
41. Sinkovics, Cs., Seress, G., **Pipoly I.**, Vincze, E., Liker, A. (2019): Szezonális és élőhelyi különbségek széncinegék (*Parus major*) utódgondozó viselkedésében, 7. Szünzoológiai Szimpózium, (Budapest) – magyar nyelvű előadás

Poster presentations

1. **Pipoly I.**, Bókony, V., Liker, A. (2009): Időjárás tényezők hatása házi verebek (*Passer domesticus*) fiókaetelési viselkedésére, XI. Magyar Etológiai Társaság Kongresszus (Göd) – magyar nyelvű poszter
2. Seress, G., Bókony, V., **Pipoly I.**, Liker, A. (2010): Morphological and reproductive differences between house sparrows (*Passer domesticus*) in relation to urbanization. Szünzoológiai Szimpózium, Magyar Természettudományi Múzeum (Budapest) – poszter angol nyelven
3. Seress, G., Bókony, V., **Pipoly I.**, Liker, A. (2010): Morphological and reproductive differences between house sparrows (*Passer domesticus*) in relation to urbanization. 11th Student Conference on Conservation Science, University of Cambridge (Cambridge, Egyesült Királyság) – poszter angol nyelven
4. **Pipoly I.**, Bókony, V., Seress, G., Szabó, K., Liker, A. (2013): Effects of weather conditions on the reproductive success of House Sparrows, "Science for Sustainability" International Conference for PhD Students, Nyugat-Magyarországi Egyetem (Győr) – poszter angol nyelven

5. **Pipoly, I.**, Bókony, V., Seress, G., Szabó, K., Liker, A. (2013): Effects of extreme meteorological conditions on reproductive success in a temperate-breeding songbird, "Climate Change and Nature Conservation in Europe" Konferencia (Bonn, Németország) – poszter angol nyelven
6. **Pipoly, I.**, Bókony, V., Seress, G., Szabó, K., Liker, A. (2013): Effects of extreme meteorological events on reproductive success in temperate-breeding House Sparrows (*Passer domesticus*, L.), "9th European Ornithologist Union" Conference (Norwich, Egyesült Királyság) – poszter angol nyelven
7. Seress, G., Papp, S., **Pipoly, I.**, Preiszner, B., Vincze, E., Bókony, V., Liker, A. (2014): Size matters: effects of habitat urbanization on nestling provisioning in two sparrow species, "7th European Conference on Behavioural Biology" (Prága, Cseh Köztársaság) – poszter angol nyelven
8. Hammer, T., Papp, S., **Pipoly, I.**, Preiszner, B., Seress, G., Vincze, E., Liker, A., Bókony, V. (2014): Effects of habitat urbanization on the breeding success of great tits (*Parus major*), "7th European Conference on Behavioural Biology" (Prága, Cseh Köztársaság) – poszter angol nyelven
9. Hammer, T., Papp, S., Seress, G., **Pipoly, I.**, Preiszner, B., Vincze, E., Liker, A. (2015): Rádiótelemetriás jelölés hatása a széncinegék (*Parus major*) viselkedésére, XVII. Magyar Etológiai Társaság Kongresszus (Dobogókő) – magyar nyelvű poszter
10. Vincze, E., Németh, B., **Pipoly, I.**, Papp, S., Preiszner, B., Seress, G., Hammer, T., Bókony, V., Liker, A. (2015): Anti-predator responses of Great Tits *Parus major* in urban and rural habitats, 10th Conference of the European Ornithologist's Union (Badajoz, Spanyolország) – poszter angol nyelven
11. Seress, G., Hammer, T., Papp, S., **Pipoly, I.**, Preiszner, B., Vincze, E., Liker, A. (2015): The effects of nest-trapping and video-recording on breeding birds' behaviour, 10th Conference of the European Ornithologist's Union (Badajoz, Spanyolország) – poszter angol nyelven
12. Sinkovics, Cs., Seress, G., **Pipoly, I.**, Hammer, T., Papp, S., Preiszner, B., Vincze, E., Liker, A. (2016): Differences in composition and biomass of nestlings' food between urban and forest great tits (*Parus major*), Student Conference on Conservation Science (Tihany) – poszter angol nyelven
13. Bukor, B., Bókony, V., Hammer, T., Papp, S., **Pipoly, I.**, Preiszner, B., Seress, G., Sinkovics, Cs., Vincze, E., Liker, A. (2016): Éven belüli és évek közötti újráfészkelés gyakorisága erdei és városi széncinege populációkban, XVIII. Magyar Etológiai Társaság Kongresszus (Debrecen) – magyar nyelvű poszter
14. Hammer T., Seress, G., Vincze, E., Preiszner, B., Papp, S., Bókony, V., **Pipoly, I.**, Sinkovics, Cs., Evans. K.L., Liker, A. (2016): Rovarevő madarak táplálékbázisának összehasonlítása városi és erdei élőhelyek között, XVIII. Magyar Etológiai Társaság Kongresszus (Debrecen) – magyar nyelvű poszter

15. **Pipoly I.**, Szabó K., Bókony V., Hammer T., Papp S., Preiszner B., Seress G., Vincze E., Liker A. (2018): Extra-pár utódok gyakorisága városi és erdei széncinege (*Parus major*) populációkban, 1. Urbanizációs Ökológia Konferencia (Veszprém) – magyar nyelvű poszter

13. References

- Adkins-Regan, E. & Reeve, H.K. (2014) Sexual dimorphism in body size and the origin of sex-determination systems. *The American Naturalist*, **183**, 519–536.
- Amemiya, C.T., Alföldi, J., Lee, A.P. et al. (2013) The African coelacanth genome provides insights into tetrapod evolution. *Nature*, **496**, 311–6.
- Anderson, T. (2006) *Biology of the Ubiquitous House Sparrow: From Genes to Populations*. Oxford University Press.
- Anderson, D.J., Reeve, J. & Bird, D. (1997) Sexually dimorphic eggs, nestling growth and sibling competition in American kestrels *Falco sparverius*. *Functional Ecology*, **11**, 331–335.
- Andreu, J. & Barba, E. (2006) Breeding dispersal of Great Tits *Parus major* in a homogeneous habitat: Effects of sex, age, and mating status. *Ardea*, **94**, 45–58.
- Arad, Z., Horowitz, M., Eylath, U. & Marder, J. (1989) Osmoregulation and body fluid compartmentalization in dehydrated heat-exposed pigeons. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, **257**, 377–382.
- Arbuckle, J.L. (2011) *IBM SPSS Amos 20 User 's Guide*. Amos Development Corporation.
- Arendt, J.D., Reznick, D.N. & López-Sepulcre, A. (2014) Replicated origin of female-biased adult sex ratio in introduced populations of the Trinidadian Guppy (*Poecilia reticulata*). *Evolution*, **68**, 2343–2356.
- Ashman, T.-L., Bachtrog, D., Blackmon, H., e al. (2014) Tree of Sex: A database of sexual systems. *Scientific Data*, **1**, 561–572.
- Bachtrog, D. (2006) A dynamic view of sex chromosome evolution. *Current Opinion in Genetics & Development*, **16**, 578–585.
- Bachtrog, D., Mank, J.E., Peichel, C.L., Kirkpatrick, M., Otto, S.P., Ashman, T.-L., Hahn, M.W., Kitano, J., Mayrose, I., Ming, R., Perrin, N., Ross, L., Valenzuela, N. & Vamosi, J.C. (2014) Tree of Sex Consortium: Sex determination: why so many ways of doing it? *PLoS biology*, **12**, e1001899.
- Badyaev, A., Whittingham, L. & Hill, G. (2001) The evolution of sexual size dimorphism in the house finch. III. Developmental basis. *Evolution*, **55**, 176–189.
- Bailly, J., Scheifler, R., Berthe, S., Clément-Demange, V.-A., Leblond, M., Pasteur, B. & Faivre, B. (2016) From eggs to fledging: negative impact of urban habitat on reproduction in two tit species. *Journal of Ornithology*, **157**, 377–392.
- Barluenga, M., Barbosa, A. & Moreno, E. (2000) Social relationships due to sex, age and morphology in Great Tits *Parus major* wintering in a mountainous habitat of Central Spain. *Ardeola*, **47**, 19–27.
- Barrett, E. & Richardson, D. (2011) Sex differences in telomeres and lifespan. *Aging cell*, **10**, 913–921.
- Bates, D., Maechler, M. & Bolker, B. (2011) Package ‘lme4.’
- Bell, S.C., Owens, I.P.F. & Lord, A.M. (2014) Quality of breeding territory mediates the influence of paternal quality on sex ratio bias in a free-living bird population. *Behavioral Ecology*, **25**, 352–358.
- Benito, M.M. & González-Solís, J. (2007) Sex ratio, sex-specific chick mortality and sexual size dimorphism in birds. *Journal of evolutionary biology*, **20**, 1522–30.
- Bessa-Gomes, C., Legendre, S. & Clobert, J. (2004) Allee effects, mating systems and the extinction risk in populations with two sexes. *Ecology Letters*, **7**, 802–812.

- Bi, P., Williams, S., Loughnan, M., Lloyd, G., Hansen, A., Kjellstrom, T., Dear, K. & Saniotis, A. (2011) The effects of extreme heat on human mortality and morbidity in Australia: Implications for public health. *Asia-Pacific Journal of Public Health*, **23**.
- Biard, C., Brischoux, F., Meillère, A., Michaud, B., Nivière, M., Ruault, S., Vaugoyeau, M. & Angelier, F. (2017) Growing in Cities: An Urban Penalty for Wild Birds? A Study of Phenotypic Differences between Urban and Rural Great Tit Chicks (*Parus major*). *Frontiers in Ecology and Evolution*, **5**.
- Birnie-Gauvin, K., Peiman, K.S., Gallagher, A.J., Bruijn, R. de & Cooke, S.J. (2016) Sublethal consequences of urban life for wild vertebrates. *Environmental Reviews*, **24**, 426–425.
- Bize, P., Roulin, A., Tella, J.L. & Richner, H. (2005) Female-biased mortality in experimentally parasitized Alpine Swift *Apus melba* nestlings. *Functional Ecology*, **19**, 405–413.
- Blumstein, D.T., Geffroy, B., Samia, D.S.M. & Bessa, E. (2017) *Ecotourism's Promise and Peril : A Biological Evaluation*. Springer.
- Bókony, V., Kövér, S., Nemesházi, E., Liker, A. & Székely, T. (2017) Climate-driven shifts in adult sex ratios via sex reversals: the type of sex determination matters. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **in press**.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.S. (2008) Generalized linear mixed models : a practical guide for ecology and evolution. *Trends in Ecology and Evolution*, **24**, 127–135.
- Bonderud, E.S., Otter, K.A., Murray, B.W., Marini, K.L.D., Burg, T.M. & Reudink, M.W. (2017) Effects of parental condition and nesting habitat on sex allocation in the mountain chickadee. *Behaviour*, **154**, 1101–1121.
- Booksmythe, I., Mautz, B., Davis, J., Nakagawa, S. & Jennions, M.D. (2017) Facultative adjustment of the offspring sex ratio and male attractiveness: a systematic review and meta-analysis. *Biol. Rev. Biological Reviews*, **92**, 108–134.
- Both, C., Bouwhuis, S., Lessells, C.M. & Visser, M.E. (2006) Climate change and population declines in a long-distance migratory bird. *Nature*, **441**, 81–3.
- Bouvier, J.C., Boivin, T., Charmantier, A., Lambrechts, M. & Lavigne, C. (2016) More daughters in a less favourable world: Breeding in intensively-managed orchards affects tertiary sex-ratio in the great tit. *Basic and Applied Ecology*, **17**, 638–647.
- Bowers, E.K., Sakaluk, S.K. & Thompson, C.F. (2011) Adaptive Sex Allocation in Relation to Hatching Synchrony and Offspring Quality in House Wrens. *The American Naturalist*, **177**, 617–629.
- Brans, K., Jansen, M., Vanoverbeke, J., Tüzün, N., Stoks, R. & De Meester, L. (2017) The heat is on: genetic adaptation to urbanization mediated by thermal tolerance and body size. *Global Change Biology*, **23**, 5218–5227.
- Brzek, P., Kohl, K., Caviades-Vidal, E. & Karasov, W.H. (2009) Developmental adjustments of house sparrow (*Passer domesticus*) nestlings to diet composition. *The Journal of experimental biology*, **212**, 1284–93.
- Buckley, L.B. & Huey, R.B. (2016) Temperature extremes: geographic patterns, recent changes, and implications for organismal vulnerabilities. *Global Change Biology*, **22**, 3829–3842.
- Burfield, I.J. & van Bommel, F. (2004) *Birds in Europe: Population Estimates, Trends and Conservation Status (Birdlife Conservation)*. BirdLife International.

- Burley, N. (1981) Sex Ratio Manipulation and Selection for Attractiveness. *Science*, **211**, 721–722.
- Burley, N. (1986) Sex-Ratio Manipulation in Color-Banded Populations of Zebra Finches. *Evolution*, **40**, 1191–1206.
- Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach*, 2nd ed.
- Burt, A. & Trivers, R. (2008) *Genes in Conflict - The Biology of Selfish Genetic Elements*, 1st ed. Harvard University Press, Cambridge.
- Calosi, P., Bilton, D.T. & Spicer, J.I. (2008) Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biology Letters*, **4**, 99–102.
- Ceballos, G., Ehrlich, P.R. & Dirzo, R. (2017) Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. *Proceedings of the National Academy of Sciences*, **114**, 6089–6096.
- Chace, J.F. & Walsh, J.J. (2006) Urban effects on native avifauna: a review. *Landscape and Urban Planning*, **74**, 46–69.
- Chalfoun, A.D. & Martin, T.E. (2007) Latitudinal variation in avian incubation attentiveness and a test of the food limitation hypothesis. *Animal Behaviour*, **73**, 579–585.
- Chamberlain, D.E., Cannon, A.R., Toms, M.P., Leech, D.I., Hatchwell, B.J. & Gaston, K.J. (2009) Avian productivity in urban landscapes: A review and meta-analysis. *Ibis*, **151**, 1–18.
- Charmantier, A., Demeyrier, V., Lambrechts, M., Perret, S. & Grégoire, A. (2017) Urbanization Is Associated with Divergence in Pace-of-Life in Great Tits. *Frontiers in Ecology and Evolution*, **5**.
- Chen, H., Wang, J., Li, Q., Yagouti, A., Lavigne, E., Foty, R., Burnett, R.T., Villeneuve, P.J., Cakmak, S. & Copes, R. (2016) Assessment of the effect of cold and hot temperatures on mortality in Ontario, Canada: a population-based study. *CMAJ Open*, **4**, E48–E58.
- Chiari, Y., Cahais, V., Galtier, N. & Delsuc, F. (2012) Phylogenomic analyses support the position of turtles as the sister group of birds and crocodiles (Archosauria). *BMC biology*, **10**, 65.
- Chung, Y., Noh, H., Honda, Y., Hashizume, M., Bell, M.L., Guo, Y.L.L. & Kim, H. (2017) Temporal Changes in Mortality Related to Extreme Temperatures for 15 Cities in Northeast Asia: Adaptation to Heat and Maladaptation to Cold. *American Journal of Epidemiology*, **185**, 907–913.
- Cichoń, M., Sendecka, J. & Gustafsson, L. (2005) Male-biased sex ratio among unhatched eggs in great tit *Parus major*, blue tit *P. caeruleus* and collared flycatcher *Ficedula albicollis*. *Journal of Avian Biology*, **36**, 386–390.
- Clutton-Brock, T.H. (2016) *MammalsocietiesTCB*, 1st ed. Wiley-Blackwell.
- Clutton-Brock & Iason. (1986) Sex ratio variation in mammals. *The Quarterly Review of Biology*, **61**, 339–374.
- Cockburn, A., Scott, M.P. & Dickman, C.R. (1985) Sex ratio and intrasexual kin competition in mammals. *Oecologia*, **66**.
- Cohen, J. (1988) *Statistical Power Analysis for the Behavioural Sciences*, 2nd ed. Lawrence Erlbaum Associates, New Jersey.
- Conrey, R.Y., Skagen, S.K., Yackel Adams, A.A. & Panjabi, A.O. (2016) Extremes of heat, drought and precipitation depress reproductive performance in shortgrass prairie passerines. *Ibis*, **158**, 614–629.

- Cordero, P.J., Griffith, S.C., Aparicio, J.M. & Parkin, D.T. (2000) Sexual dimorphism in house sparrow eggs. *Behavioral Ecology and Sociobiology*, **48**, 353–357.
- Cordero, P.J., Vinuela, J., Aparicio, J.M. & Veiga, J.P. (2001) Seasonal variation in sex ratio and sexual egg dimorphism favouring daughters in first clutches of the spotless starling. *Journal of Evolutionary Biology*, **14**, 829–834.
- Cox, B., Gasparrini, A., Catry, B., Delcloo, A., Bijmens, E., Vangronsveld, J. & Nawrot, T.S. (2016) Mortality related to cold and heat. What do we learn from dairy cattle? *Environmental Research*, **149**, 231–238.
- Crick, H.Q.P. (2004) The impact of climate change on birds. *Ibis*, **146**, 48–56.
- Crick, H.Q.P. & Sparks, T.H. (2006) Changes in the phenology of breeding and migration in relation to global climate change. *Acta Zoologica Sinica*, **52**, 154–157.
- Crooks, K.R., Suarez, A. V & Bolger, D.T. (2004) Avian assemblages along a gradient of urbanization in a highly fragmented landscape. *Biological Conservation*, **115**, 451–462.
- Csörgő, T., Harnos, A. & Kovács, S. (2009) A klímaváltozás hatásainak vizsgálata hosszútávú madárgyűrűzési adatsorok elemzésével. *Természetvédelmi Közlemények*, **15**, 1–12.
- Davies, N.B., Krebs, J.R. & West, S.A. (2012) *An Introduction to Behavioural Ecology*, 4th ed. Wiley-Blackwell, Oxford.
- Dawson, R.D. (2008) Timing of breeding and environmental factors as determinants of reproductive performance of tree swallows. *Canadian Journal of Zoology*, **86**, 843–850.
- Deeming, D.C. (2008) Avian brood patch temperature: Relationships with female body mass, incubation period, developmental maturity and phylogeny. *Journal of Thermal Biology*, **33**, 345–354.
- Deschenes, O. & Moretti, E. (2007) *Extreme Weather Events, Mortality and Migration*.
- Dhondt, A.A. (1970) The sex ratio of nestling great tits. *Bird Study*, **17**, 282–286.
- Dhondt, A.A., Eyckerman, R., Moermans, R. & Huble, J. (1984) Habitat and laying date of great and blue tit *Parus major* and *Parus caeruleus*. *Ibis*, **126**, 388–397.
- Diamond, S.E., Chick, L., Perez, A.B.E., Strickler, S.A. & Martin, R.A. (2017) Rapid evolution of ant thermal tolerance across an urban-rural temperature cline. *Biological Journal of the Linnean Society*, **121**, 248–257.
- Dijkstra, C., Riedstra, B., Dekker, A., Goerlich, V.C., Daan, S. & Groothuis, T.G.G. (2010) An adaptive annual rhythm in the sex of first pigeon eggs. *Behavioral ecology and sociobiology*, **64**, 1393–1402.
- Dingemanse, N.J., Dochtermann, N. & Wright, J. (2010) A method for exploring the structure of behavioural syndromes to allow formal comparison within and between data sets. *Animal Behaviour*, **79**, 439–450.
- Dingemanse, N.J. & De Goede, P. (2004) The relation between dominance and exploratory behavior is context-dependent in wild great tits. *Behavioral Ecology*, **15**, 1023–1030.
- Donald, P.F. (2007) Adult sex ratios in wild bird populations. *Ibis*, **149**, 671–692.
- van Doorn, G.S. & Kirkpatrick, M. (2010) Transitions between male and female heterogamety caused by sex-antagonistic selection. *Genetics*, **186**, 629–645.
- Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J.R.G., Gruber, B., Lafourcade, B., Leitão, P.J., Münkemüller, T., McClean, C., Osborne, P.E., Reineking, B., Schröder,

- B., Skidmore, A.K., Zurell, D. & Lautenbach, S. (2012) Collinearity : a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, **35**, 1–20.
- Du, W., Robbins, T.R., Warner, D.A., Langkilde, T. & Shine, R. (2014) Latitudinal and seasonal variation in reproductive effort of the eastern fence lizard (*Sceloporus undulatus*). *Integrative Zoology*, **9**, 360–371.
- Edwards, A.M. & Cameron, E.Z. (2014) Forgotten fathers: paternal influences on mammalian sex allocation. *Trends in ecology & evolution*, **29**, 158–64.
- Elkins, N. (2004) *Weather and Bird Behaviour*, Third edit. T & A D Poyser, London.
- European, Environment & Agency. (2012) *Climate Change, Impacts and Vulnerability in Europe*.
- Evans, B.J., Pyron, R.A. & Wiens, J.J. (2012) Polyploidization and Sex Chromosome Evolution in Amphibians. *Polyploidy and Genome Evolution* (eds P.S. Soltis), & D.E. Soltis), pp. 385–410. Springer Berlin Heidelberg, Berlin, Heidelberg.
- Ewen, J.G., Cassey, P., Møller, A.P., Curie, M., Cedex, P. & Supe, N. (2004) Facultative primary sex ratio variation : a lack of evidence in birds ? *Proceedings of The Royal Society B*, **271**, 1277–1282.
- Field, C., Barros, V., Stocker, T. & Dahe, Q. (2012) Managing the risks of extreme events and disasters to advance climate change adaptation. *IPCC Report*, 582.
- Field, D.L., Pickup, M. & Barrett, S.C.H. (2013) Comparative analyses of sex-ratio variation in dioecious flowering plants. *Evolution*, **67**, 661–672.
- Fisher, R.A. (1930) *The Genetic Theory of Natural Selection*. Oxford University Press, Oxford.
- Freckleton, R.P., Harvey, P.H. & Pagel, M. (2002) Phylogenetic analysis and comparative data: a test and review of evidence. *The American Naturalist*, **160**, 712–26.
- Fridolfsson, A.K. & Ellegren, H. (1999) A simple and universal method for molecular sexing of non-ratite birds. *Journal of Avian Biology*, **30**, 116–121.
- Fritz, S.A., Bininda-Emonds, O.R.P. & Purvis, A. (2009) Geographical variation in predictors of mammalian extinction risk: big is bad, but only in the tropics. *Ecology Letters*, **12**, 538–549.
- Gabriel, K.M.A. & Endlicher, W.R. (2011) Urban and rural mortality rates during heat waves in Berlin and Brandenburg, Germany. *Environmental Pollution*, **159**, 2044–2050.
- Le Galliard, J.-F., Fitze, P.S., Ferrière, R. & Clobert, J. (2005) Sex ratio bias, male aggression, and population collapse in lizards. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 18231–6.
- Gardner, M.G., Hugall, A.F., Donnellan, S.C., Hutchinson, M.N. & Foster, R. (2008) Molecular systematics of social skinks : phylogeny and taxonomy of the Egernia group (Reptilia : Scincidae). *Zoological Journal of the Linnean Society*, **154**, 781–794.
- Gardner, J.L., Rowley, E., De Rebeira, P., De Rebeira, A. & Brouwer, L. (2017) Effects of extreme weather on two sympatric australian passerine bird species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **372**.
- Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., Diaz, D., Harmelin, J.G., Gambi, M.C., Kersting, D.K., Ledoux, J.B., Lejeune, C., Linares, C., Marschal, C., Pérez, T., Ribes, M., Romano, J.C., Serrano, E., Teixido, N., Torrents, O., Zabala, M., Zuberer, F. & Cerrano, C. (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: Effects of the 2003 heat wave. *Global Change Biology*, **15**, 1090–1103.

- Gasparri, A. & Armstrong, B. (2011) The impact of heat waves on mortality. *Epidemiology (Cambridge, Mass.)*, **22**, 68–73.
- Geerts, A.N., Vanoverbeke, J., Vanschoenwinkel, B., Van Doorslaer, W., Feuchtmayr, H., Atkinson, D., Moss, B., Davidson, T.A., Sayer, C.D. & De Meester, L. (2015) Rapid evolution of thermal tolerance in the water flea *Daphnia*. *Nature Climate Change*, **5**, 665–668.
- Gemmell, N.J. & Akayima, S. (1996) An efficient method for the extraction of DNA from vertebrate tissues. *Trends in Genetics*, **12**, 338–339.
- Gil, D. & Brunn, H. (eds). (2013) *Avian Urban Ecology: Behavioural and Physiological Adaptations*. Oxford University p, Oxford.
- Gładalski, M., Bańbura, M., Kaliński, A., Markowski, M., Skwarska, J., Wawrzyniak, J., Zieliński, P. & Bańbura, J. (2018) Hatching delays in great tits and blue tits in response to an extreme cold spell: a long-term study. *International Journal of Biometeorology*, **62**, 1437–1445.
- Göth, A. & Booth, D.T. (2005) Temperature-dependent sex ratio in a bird. *Biology letters*, **1**, 31–3.
- Grace, J.B., Anderson, M., Olf, H. & Scheiner, S.M. (2010) On the specification of structural equation models for ecological systems. *Ecological Monographs*, **80**, 67–87.
- Griffiths, R., Double, M.C., Orr, K. & Dawson, R.J.G. (1998) A DNA test to sex most birds. *Molecular Biology*, **7**, 1071–1075.
- Griskevicius, V., Tybur, J.M., Ackerman, J.M., Delton, A.W., Robertson, T.E. & White, A.E. (2012) The financial consequences of too many men: sex ratio effects on saving, borrowing, and spending. *Journal of personality and social psychology*, **102**, 69–80.
- Guillon, J.-M., Guery, L., Hulin, V. & Girondot, M. (2012) A large phylogeny of turtles (Testudines) using molecular data. *Contributions to Zoology*, **81**.
- Gullett, P.R., Hatchwell, B.J., Robinson, R.A. & Evans, K.L. (2015) Breeding season weather determines long-tailed tit reproductive success through impacts on recruitment. *Journal of Avian Biology*, **46**, 441–451.
- Haldane, J.B. (1922) Sex-ratio and unisexual sterility in hybrid animals. *Journal of Genetics*, **12**, 101–109.
- Hamilton, W.D. (1967) Extraordinary sex ratios. *Science*, **156**, 477–488.
- Han, X. & Baker, D.H. (1993) Effects of sex, heat stress, body weight, and genetic strain on the dietary lysine requirement of broiler chicks. *Poultry Science*, **72**, 701–708.
- Hansen, J., Sato, M. & Ruedy, R. (2012) Perception of climate change. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 1–9.
- Hardy, Ian, C. (2010) Sex Allocation, Sex Ratios and Reproduction. *Encyclopedia of Animal Behavior* pp. 146–151. IOP Publishing.
- Hasselquist, D. & Kempenaers, B. (2002) Parental care and adaptive brood sex ratio manipulation in birds. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **357**, 363–72.
- Healy, K., Guillerme, T., Finlay, S., Kane, A., Kelly, S.B.A., McClean, D., Kelly, J., Donohue, I., Jackson, A.L., Cooper, N., B, P.R.S., Kelly, B.A. & Kelly, D.J. (2014) and mammals Ecology and mode-of-life explain lifespan variation in birds and mammals Author for correspondence : *Proceedings of the Royal Society B*, **281**.

- Heaviside, C., Mitchell, D., Wallom, D., Allen, M., Vardoulakis, S., Huntingford, C., Masato, G., Bowery, A., P Guillod, B. & Frumhoff, P. (2016) Attributing human mortality during extreme heat waves to anthropogenic climate change. *Environmental Research Letters*, **11**, 074006.
- Hegyi, G. & Nagy, G. (2012) Reduced compensatory growth capacity in mistimed broods of a migratory passerine. *Oecologia*.
- Hepp, G.R., Kennamer, R. a. & Johnson, M.H. (2006) Maternal effects in Wood Ducks: incubation temperature influences incubation period and neonate phenotype. *Functional Ecology*, **20**, 308–314.
- Hinsley, S., Hill, R., Bellamy, P., Harrison, N., Speakman, J., Wilson, A. & Ferns, P. (2008) Effects of structural and functional habitat gaps on breeding woodland birds: working harder for less. *Landscape Ecology*, **23**, 615–626.
- Hochachka, W.M. & Smith, J. (1991) Determinants and consequences of nestling condition in song sparrows. *The Journal of Animal Ecology*, **60**, 995–1008.
- Hoi, H., Vaclav, R. & Slobodova, D. (2003) Postmating sexual selection in house sparrows: can females estimate good fathers according to their early paternal effort? *Folia zoologica*, **52**, 299–308.
- Højsgaard, S., Halekoh, U. & Yan, J. (2006) The R Package geepack for Generalized Estimating Equations. *Journal of Statistical Software*, **15**, 1–11.
- Holm, E. & Edney, E.B. (1973) Daily activity of Namib desert arthropods in relation to climate. *Ecological Monographs*, **54**, 45–56.
- Horak, P. (1993) Low fledging success of urban Great Tits. *Ornis Fennica*, **70**, 168–172.
- Hörak, P. & Lebreton, J.-D. (1998) Survival of adult Great Tits *Parus major* in relation to sex and habitat; a comparison of urban and rural populations. *Ibis*, **140**, 205–209.
- Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous Inference in General Parametric Models. *Biometrical Journal*, **50**, 346–363.
- Hough, J., Immler, S., Barrett, S.C.H. & Otto, S.P. (2013) Evolutionarily Stable Sex Ratios And Mutation Load. *Evolution*, **67**, 1915–1925.
- Howe, H. (1977) Sex-ratio adjustment in the common grackle. *Science*, **198**, 744–746.
- Hungarian Meteorological Service. (2013) URL www.met.hu
- Iverson, J.B., Balgooyen, C.P., Byrd, K.K. & Lyddan, K.K. (1993) Latitudinal variation in egg and clutch size in turtles. *Canadian Journal of Zoology*, **71**, 2448–2461.
- Jaenike, J. (2001) Sex chromosome meiotic drive. *Annual Review of Ecology and Systematics*, **32**, 25–49.
- Jentsch, A. & Beierkuhnlein, C. (2008) Research frontiers in climate change: Effects of extreme meteorological events on ecosystems. *Comptes Rendus Geoscience*, **340**, 621–628.
- Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K. & Mooers, A.O. (2012) The global diversity of birds in space and time. *Nature*, **491**, 444–448.
- Jiguet, F., Brotons, L. & Devictor, V. (2011) Community responses to extreme climatic conditions. *Current Zoology*, **57**, 406–413.
- Jiguet, F., Julliard, R., Thomas, C.D., Dehorter, O., Newson, S.E. & Couvet, D. (2006) Thermal range predicts bird population resilience to extreme high temperatures. *Ecology Letters*, **9**, 1321–1330.
- Jones, K.E., Bielby, J., Cardillo, M., Fritz, S.A., O'Dell, J., Orme, C.D.L., Safi, K., Sechrest, W., Boakes, E.H., Carbone, C., Connolly, C., Cutts, M.J., Foster, J.K., Grenyer, R., Habib, M., Christopher A.

- Plaster, S.A.P., Rigby, E.A., Rist, J., Teacher, A., Bininda-Emonds, O.R.P., Gittleman, J.L., Mace, G.M. & Purvis, A. (2009) PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. *Ecology*, **90**.
- Jones, A.G., Small, C.M., Paczolt, K.A. & Ratterman, N.L. (2010) A practical guide to methods of parentage analysis. *Molecular Ecology Resources*, **10**, 6–30.
- Jongepier, E. (2011) *Reptilian Adult Sex Ratios Are Biased Towards the Homogametic Sex*.
- Julliard, R. (2000) Sex-specific dispersal in spatially varying environments leads to habitat-dependent evolutionarily stable offspring sex ratios. *Behavioral Ecology*, **11**, 421–428.
- Kabasakal, B. & Albayrak, T. (2012) Offspring sex ratios and breeding success of a population of the great tit, *Parus major*. *Zoology in the Middle East*, **57**, 27–34.
- Kaiser, A., Merckx, T. & Van Dyck, H. (2016) The Urban Heat Island and its spatial scale dependent impact on survival and development in butterflies of different thermal sensitivity. *Ecology and Evolution*, **6**, 4129–4140.
- Kalinowski, S.T., Taper, M.L. & Marshall, T.C. (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099–1106.
- Kalmbach, E., Nager, R.G., Griffiths, R. & Furness, R.W. (2001) Increased reproductive effort results in male-biased offspring sex ratio: An experimental study in a species with reversed sexual size dimorphism. *Proceedings of the Royal Society B: Biological Sciences*, **268**, 2175–2179.
- Katz, R.W. & Brown, B.G. (1992) Extreme Events in a Changing Climate: Variability is More Important Than Averages. *Climatic Change*, **21**, 289–302.
- Kilner, R. (1998) Primary and secondary sex ratio manipulation by zebra finches. *Animal behaviour*, **56**, 155–64.
- Kingsolver, J.G., Woods, H.A., Kingsolver, J.G. & Woods, H.A. (1997) Thermal Sensitivity of Growth and Feeding in *Manduca sexta* Caterpillars. *Physiological Zoology*, **70**, 631–638.
- Kirkpatrick, M. (2017) The evolution of genome structure by natural and sexual selection. *Journal of Heredity*, **108**, 3–11.
- Knape, J. & de Valpine, P. (2011) Effects of weather and climate on the dynamics of animal population time series. *Proceedings of The Royal Society B*, **278**, 985–92.
- Kokko, H. & Jennions, M.D. (2008) Parental investment, sexual selection and sex ratios. *Journal of evolutionary biology*, **21**, 919–48.
- Kölliker, M., Heeb, P., Werner, I., Mateman, A.C., Lessells, C.M. & Richner, H. (1999) Offspring sex ratio is related to male body size in the great tit (*Parus major*). *Behavioral Ecology*, **10**, 68–72.
- Komdeur, J. (1996) Facultative sex ratio bias in the offspring of Seychelles warblers. *Proceedings. Biological sciences*, **263**, 661–666.
- Komdeur, J. & Pen, I. (2002) Adaptive sex allocation in birds: the complexities of linking theory and practice. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **357**, 373–380.
- Korpimäki, E., May, C.A., Parkin, D.T., Wetton, J.H. & Wiehn, J. (2000) Environmental- and parental condition-related variation in sex ratio of kestrel broods. *Journal of Avian Biology*, **31**, 128–134.
- Kovács, S., Csörgő, T., Harnos, A., Fehérvári, P. & Nagy, K. (2010) Change in migration phenology and biometrics of two conspecific *Sylvia* species in Hungary. *Journal of Ornithology*, **152**, 365–373.

- Krause, J.S., Chmura, H.E., Wingfield, J.C., Lisovski, S., Word, K.R., González-Gómez, P.L. & Pérez, J.H. (2017) How birds cope physiologically and behaviourally with extreme climatic events. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **372**, 20160140.
- Kruuk, L.E.B., Osmond, H.L. & Cockburn, A. (2015) Contrasting effects of climate on juvenile body size in a Southern Hemisphere passerine bird. *Global Change Biology*, **21**, 2929–2941.
- De Laet, J. & Summers-Smith, J.D. (2007) The status of the urban house sparrow *Passer domesticus* in north-western Europe: A review. *Journal of Ornithology*, **148**, 275–278.
- Landsberg, H.E. (1981) *The Urban Climate*.
- Lavergne, S., Mouquet, N., Thuiller, W. & Ronce, O. (2010) Biodiversity and Climate Change: Integrating Evolutionary and Ecological Responses of Species and Communities. *Annual Review of Ecology, Evolution, and Systematics*, **41**, 321–350.
- Lee, K.P. & Roh, C. (2010) Temperature-by-nutrient interactions affecting growth rate in an insect ectotherm. *Entomologia Experimentalis et Applicata*, **136**, 151–163.
- Lemoine, N.P., Capdevielle, J.N. & Parker, J.D. (2015) Effects of in situ climate warming on monarch caterpillar (*Danaus plexippus*) development. *PeerJ*, 1–10.
- Lenth, R. V. (2018) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.2.3.
- Lessells, C.M. & Boag, P.T. (1987) Unrepeatable Repeatabilities? : A Common Mistake. *The Auk*, **104**, 116–121.
- Lessells, C.M., Mateman, A.C. & Visser, J. (1996) Great Tit Hatchling Sex Ratios. *Journal of Avian Biology*, **27**, 135.
- Ligon, R.A. & Hill, G.E. (2010) Sex-biased parental investment is correlated with mate ornamentation in eastern bluebirds. *Animal Behaviour*, **79**, 727–734.
- Liker, A., Freckleton, R.P. & Székely, T. (2013) The evolution of sex roles in birds is related to adult sex ratio. *Nature communications*, **4**, 1587.
- Liker, A., Freckleton, R.P. & Székely, T. (2014) Divorce and Infidelity Are Associated with Skewed Adult Sex Ratios in Birds. *Current Biology*, **24**, 880–884.
- Liker, A., Papp, Z., Bókony, V. & Lendvai, Á.Z. (2008) Lean birds in the city: Body size and condition of house sparrows along the urbanization gradient. *Journal of Animal Ecology*, **77**, 789–795.
- Liker, A. & Székely, T. (2005) Mortality costs of sexual selection and parental care in natural populations of birds. *Evolution*, **59**, 890–897.
- Linden, M., Gustafsson, L. & Part, T. (1992) Selection on Fledging Mass in the Collared Flycatcher and the Great Tit. *Ecology*, **73**, 336–343.
- Lindquist, S. (1986) The heat-shock response. *Annual Review of Biochemistry*, **55**, 1151–1191.
- Lislevand, T., Figuerola, J. & Székely, T. (2007) Avian body sizes in relation to fecundity, mating system, display behaviour and resource sharing. *Ecology*, **88**, 1605.
- Lovich, J.E., Gibbons, J.W. & Agha, M. (2014) Does the timing of attainment of maturity influence sexual size dimorphism and adult sex ratio in turtles? *Biological Journal of the Linnean Society*, **112**, 142–149.
- Maddison, W.P. & Maddison, D.R. (2011) Mesquite: a modular system for evolutionary analysis.

- Mank, J.E. (2013) Sex chromosome dosage compensation: definitely not for everyone. *Trends in Genetics*, **29**, 677–683.
- Marrot, P., Garant, D. & Charmantier, A. (2017) Multiple extreme climatic events strengthen selection for earlier breeding in a wild passerine. *Philos Trans R Soc Lond B Biol Sci*, **this issue**.
- Martin, T.E. (1987) Food as a limit on breeding birds: a life-history perspective. *Annual Review of Ecology, Evolution, and Systematics*, **18**, 453–487.
- Martins, T.L.F. (2004) Sex-specific growth rates in zebra finch nestlings: a possible mechanism for sex ratio adjustment. *Behavioral Ecology*, **15**, 174–180.
- Marzluff, J.M. (2017) A decadal review of urban ornithology and a prospectus for the future. *Ibis*, **159**, 1–13.
- Massemin, S., Korpimäki, E. & Wiehn, J. (2000) Reversed sexual size dimorphism in raptors: evaluation of the hypotheses in kestrels breeding in a temporally changing environment. *Oecologia*, **124**, 26–32.
- Maxwell, S.L., Fuller, R.A., Brooks, T.M. & Watson, J.E.M. (2016) Biodiversity – The ravages of guns, nets and bulldozers. *Nature*, **536**.
- McDonald, P.G., Olsen, P.D. & Cockburn, A. (2004) Weather dictates reproductive success and survival in the Australian brown falcon *Falco berigora*. *Journal of Animal Ecology*, **73**, 683–692.
- McIntyre, N.E. (2000) Ecology of Urban Arthropods : A Review and a Call to Action. *Annals of The Entomological Society of America*, **93**, 825–835.
- McKechnie, A.E., Hockey, P.A.R. & Wolf, B.O. (2012) Feeling the heat: Australian land birds and climate change. *EMU*, **112**, 1–7.
- Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L.F., Backeljau, T., Bonte, D., Brans, K.I., Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J.M.T., Fontaneto, D., Gianuca, A.T., Govaert, L., Hendrickx, F., Higiuti, J., Lens, L., Martens, K., Matheve, H., Matthysen, E., Piano, E., Sablon, R., Schön, I., Van Doninck, K., De Meester, L. & Van Dyck, H. (2018) Body-size shifts in aquatic and terrestrial urban communities. *Nature*, **558**, 113–116.
- Meredith, R.W., Janečka, J.E., Gatesy, J., Ryder, O.A., Fisher, C.A., Teeling, E.C., Goodbla, A., Eizirik, E., Simão, T.L.L., Stadler, T., Rabosky, D.L., Honeycutt, R.L., Flynn, J.J., Ingram, C.M., Steiner, C., Williams, T.L., Robinson, T.J., Burk-Herrick, A., Westerman, M., Ayoub, N.A., Springer, M.S. & Murphy, W.J. (2011) Impacts of the Cretaceous Terrestrial Revolution and KPg extinction on mammal diversification. *Science*, **334**, 521–4.
- Mertens, J.A.L. (1977) Thermal conditions for successful breeding in Great Tits (*Parus major* L.). *Oecologia*, **28**, 31–56.
- Miller-Rushing, A.J., Høye, T.T., Inouye, D.W. & Post, E. (2010) The effects of phenological mismatches on demography. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **365**, 3177–86.
- Miranda, A.C., Schielzeth, H., Sonntag, T. & Partecke, J. (2013) Urbanization and its effects on personality traits: A result of microevolution or phenotypic plasticity? *Global Change Biology*, **19**, 2634–2644.
- Miura, I., Ohtani, H. & Ogata, M. (2012) Independent degeneration of W and Y sex chromosomes in frog *Rana rugosa*. *Chromosome Research*, **20**, 47–55.
- Mock, D.W. & Schwagmeyer, P.L. (2010) Not the nice sparrow. *The Wilson Journal of Ornithology*, **122**,

207–216.

- Møller, A.P. (2011) Behavioral and life history responses to extreme climatic conditions : Studies on a migratory songbird. *Current Zoology*, **57**, 351–362.
- Moller, A.P., Diaz, M., Flensted-Jensen, E., Grim, T., Ibáñez-Álamo, J.D., Jokimaki, J., Mänd, R., Markó, G. & Tryjanowski, P. (2012) High urban population density of birds reflects their timing of urbanization. *Oecologia*, **170**, 867–875.
- Møller, A. & Jennions, M. (2002) How much variance can be explained by ecologists and evolutionary biologists? *Oecologia*, **132**, 492–500.
- Møller, A.P., Rubolini, D. & Lehikoinen, E. (2008) Populations of migratory bird species that did not show a phenological response to climate change are declining. *Proceedings of the National Academy of Sciences*, **105**, 16195–16200.
- Moore, J. a., Kamarainen, A.M., Scribner, K.T., Mykut, C. & Prince, H.H. (2012) The effects of anthropogenic alteration of nesting habitat on rates of extra-pair fertilization and intraspecific brood parasitism in Canada Geese *Branta canadensis*. *Ibis*, **154**, 354–362.
- Morelli, F., Benedetti, Y., Ibáñez-Álamo, J.D., Jokimäki, J., Mänd, R., Tryjanowski, P. & Møller, A.P. (2016) Evidence of evolutionary homogenization of bird communities in urban environments across Europe. *Global Ecology and Biogeography*, **25**, 1284–1293.
- Morignat, E., Perrin, J.B., Gay, E., Vinard, J.L., Calavas, D. & Hénaux, V. (2014) Assessment of the impact of the 2003 and 2006 heat waves on cattle mortality in France. *PLoS ONE*, **9**.
- Morrison, C. & Hero, J.-M. (2003) Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology*, **72**, 270–279.
- Murphy, M.T. (1985) Nestling Eastern Kingbird Growth: Effects of Initial Size and Ambient Temperature. *Ecology*, **66**, 162–170.
- Naef-Daenzer, B., Widmer, F. & Nuber, M. (2001) Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. *Journal of Animal Ecology*, **70**, 730–738.
- Nakagawa, S. & Cuthill, I.C. (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological reviews of the Cambridge Philosophical Society*, **82**, 591–605.
- Nilsson, J.-Å., Molokwu, M.N. & Olsson, O. (2016) Body Temperature Regulation in Hot Environments. *PloS ONE*, **11**, 1–9.
- Nilsson, J.-Å. & Nord, A. (2018) Testing the heat dissipation limit theory in a breeding passerine. *Proceedings of The Royal Society B*, **285**, 1–8.
- Nomi, D., Yuta, T. & Koizumi, I. (2018) Seasonal change in sexual differences in nestling size and survival: A framework to evaluate sex-dependent environmental sensitivity in the wild. *Avian Research*, **9**, 1–8.
- Oddie, K.R. (2000) Size matters : competition between male and female great tit offspring. *Ecology*, **69**, 903–912.
- Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.
- Orme, A.D., Freckleton, R., Thomas, G., Petzoldt, T., Isaac, N., Pearse, W. & Orme, M.D. (2013) caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5.

- Pagel, M. (1994) Detecting correlated evolution on phylogenies: A general method for the comparative analysis of discrete characters. *Proceedings of The Royal Society B*, **255**, 37–45.
- Pagel, M. (1998) Inferring evolutionary processes from phylogenies. *Zoologica Scripta*, **26**, 331–348.
- Pagel, M. & Meade, A. (2006) A Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *American Naturalist*, **167**, 808–825.
- Palmer, G., Brereton, T., Chapman, J., Dytham, C., Fox, R., Pearce-Higgins, J., Roy, D., Hill, J. & Thomas, C. (2017) Climate change, climatic variation, and extreme biological responses. *Philosophical Transactions of the Royal Society B*, 1–33.
- Parmesan, C. (2006) Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637–669.
- Peach, W.J., Vincent, K.E., Fowler, J. a. & Grice, P. V. (2008) Reproductive success of house sparrows along an urban gradient. *Animal Conservation*, **11**, 493–503.
- Perrins, C.M. (1965) Population fluctuations and clutch-size in the Great tit *Parus major* L. *Journal of Animal Ecology*, **34**, 601–647.
- Petrie, M. & Kempnaers, B. (1998) Extra-pair paternity in birds: explaining variation between species and populations. *Trends in Ecology & Evolution*, **13**, 52–58.
- Pickett, S.T.A., Cadenasso, M.L., Grove, J.M., Boone, C.G., Groffman, P.M., Irwin, E., Kaushal, S.S., Marshall, V., McGrath, B.P., Nilon, C.H., Pouyat, R. V., Szlavecz, K., Troy, A. & Warren, P. (2011) Urban ecological systems: Scientific foundations and a decade of progress. *Journal of Environmental Management*, **92**, 331–362.
- Piedrahita, P., Meise, K., Werner, C., Krüger, O. & Trillmich, F. (2014) Lazy sons, self-sufficient daughters: are sons more demanding? *Animal Behaviour*, **98**, 69–78.
- Piepho, H.P., Büchse, A. & Emrich, K. (2003) A Hitchhiker ' s Guide to Mixed Models for Randomized Experiments. *Agronomy & Crop Science*, **189**, 310–322.
- Pike, T.W. & Petrie, M. (2003) Potential mechanisms of avian sex manipulation. *Biological Reviews of the Cambridge Philosophical Society*, **78**, 553–574.
- Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. (2013) NLME: Linear and nonlinear mixed effects models. , **Version 3.**, 1–336.
- Pipoly, I., Bókony, V. & Liker, A. (2011) Hogyan befolyásolja az időjárás a házi verebek (*Passer domesticus*) fiókaetelési viselkedését ? *Állattani Közlemények*, **96**, 97–111.
- Pokorná, M. & Kratochvíl, L. (2009) Phylogeny of sex-determining mechanisms in squamate reptiles: are sex chromosomes an evolutionary trap? *Zoological Journal of the Linnean Society*, **156**, 168–183.
- van de Pol, M., Jenouvrier, S., Cornelissen, J.H. & Visser, M.E. (2017) Behavioural , ecological and evolutionary responses to extreme climatic events: challenges and directions. *Philosophical Transactions of the Royal Society B*, **372**, 1–16.
- Pryke, S.R. & Rollins, L.A. (2012) Mothers adjust offspring sex to match the quality of the rearing environment. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 4051–4057.
- Pyron, R., Burbrink, F.T., Wiens, J.J., Anisimova, M., Hordijk, W., Gascuel, O., Valentin, F., Wallace, I., Wilm, A. & Lopez, R. (2013) A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology*, **13**, 93.
- Pyron, R.A. & Wiens, J.J. (2011) A large-scale phylogeny of Amphibia including over 2800 species, and

- a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, **61**, 543–83.
- Qi, Y., Yang, W., Lu, B. & Fu, J. (2013) Genetic evidence for male-biased dispersal in the Qinghai toad-headed agamid *Phrynocephalus vlangalii* and its potential link to individual social interactions. *Ecology and evolution*, **3**, 1219–30.
- Quinteiro-Filho, W.M., Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M.L., Sakai, M., Sá, L.R.M., Ferreira, A.J.P. & Palermo-Neto, J. (2010) Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. *Poultry Science*, **89**, 1905–1914.
- R Core Team. (2014) R: A Language and Environment for Statistical Computing.
- R Development Core Team, R. (2017) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Råberg, L., Stjernman, M. & Nilsson, J.Å. (2005) Sex and environmental sensitivity in blue tit nestlings. *Oecologia*, **145**, 496–503.
- Radford, A.N. & Blakey, J.K. (2000) Intensity of nest defence is related to offspring sex ratio in the great tit *Parus major*. *Proceedings. Biological sciences / The Royal Society*, **267**, 535–8.
- Radford, A.N., McCleery, R.H., Woodburn, R.J.W. & Morecroft, M.D. (2001) Activity patterns of parent Great Tits *Parus major* feeding their young during rainfall. *Bird Study*, **48**, 214–220.
- Reid, J.M., Monaghan, P. & Ruxton, G.D. (2000) Resource allocation between reproductive phases: the importance of thermal conditions in determining the cost of incubation. *Proceedings. Biological sciences / The Royal Society*, **267**, 37–41.
- Reid, J.M., Monaghan, P. & Ruxton, G.D. (2002) Males matter: the occurrence and consequences of male incubation in starlings (*Sturnus vulgaris*). *Behavioral Ecology and Sociobiology*, **51**, 255–261.
- Rejt, Ł., Gryczyńska-Sięmiątkowska, A., Rutkowski, R. & Malewska, A. (2005) Does egg sex ratio in urban kestrels (*Falco tinnunculus*) differ from parity? *Polish Journal of Ecology*, **53**, 545–552.
- Ringsby, T., Saether, B.-E. & Solberg, E.J. (1998) Factors affecting juvenile survival in house sparrow *Passer domesticus*. *Journal of Avian Biology*, **29**, 241–247.
- Ripley, B.D., Venables, W.N., Bates, D.M., Hornik, K., Gebhardt, A., Firth, D. & Ripley, M.B. (2013) Package “MASS.” *CRAN Repository*.
- Roberts, R.B., Ser, J.R. & Kocher, T.D. (2009) Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi Cichlid fishes. *Science*, **326**, 998–1001.
- Robine, J.M., Cheung, S.L.K., Le Roy, S., Van Oyen, H., Griffiths, C., Michel, J.P. & Herrmann, F.R. (2008) Death toll exceeded 70,000 in Europe during the summer of 2003. *Comptes Rendus - Biologies*, **331**, 171–178.
- Roche, E.A., Brown, M.B. & Brown, C.R. (2014) The effect of weather on morphometric traits of juvenile cliff swallows. *The Prairie Naturalist*, **46**, 76–87.
- Rodewald, A.D. & Gehrt, S.D. (2014) Wildlife Population Dynamics in Urban Landscapes. *Urban Wildlife Conservation: Theory and Practice* (eds R.A. McCleery, C.E. Moorman, & M.N. Peterson), pp. 117–147. Springer Science + Business Media.
- Rodríguez-Martínez, S., Carrete, M., Roques, S., Rebolo-Ifrán, N. & Tella, J.L. (2014) High urban breeding densities do not disrupt genetic monogamy in a bird species. *PloS one*, **9**, 8.
- Rodríguez, S. & Barba, E. (2016) Nestling growth is impaired by heat stress: An experimental study in a

- mediterranean great tit population. *Zoological Studies*, **55**.
- Rosenfield, R.N., Sonsthagen, S.A., Stout, W.E. & Talbot, S.L. (2015) High frequency of extra-pair paternity in an urban population of Cooper's Hawks. *Journal of Field Ornithology*, **86**, 144–152.
- Rosivall, B., Szöllösi, E., Hasselquist, D. & Török, J. (2010) Males are sensitive — sex-dependent effect of rearing conditions on nestling growth. *Behavioral Ecology and Sociobiology*, **64**, 1555–1562.
- Rosivall, B., Török, J., Hasselquist, D. & Bensch, S. (2004) Brood sex ratio adjustment in collared flycatchers (*Ficedula albicollis*): results differ between populations. *Behavioral Ecology and Sociobiology*, **56**, 346–351.
- Rousset, F. (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.
- Ruxton, G.D. & Beauchamp, G. (2008) Time for some a priori thinking about post hoc testing. *Behavioral Ecology*, **19**, 690–693.
- Sabath, N., Itescu, Y., Feldman, A., Meiri, S., Mayrose, I. & Valenzuela, N. (2016) Sex determination, longevity, and the birth and death of reptilian species. *Ecology and Evolution*, **6**, 5207–5220.
- Salido, L., Purse, B. V., Marrs, R., Chamberlain, D.E. & Shultz, S. (2012) Flexibility in phenology and habitat use act as buffers to long-term population declines in UK passerines. *Ecography*, **35**, 604–613.
- Sarre, S.D., Ezaz, T. & Georges, A. (2011) Transitions between sex-determining systems in reptiles and amphibians. *Annual review of Genomics and Human Genetics*, **12**, 391–406.
- Schacht, R., Kramer, K.L., Székely, T. & Kappeler, P.M. (2017) Adult sex ratios and reproductive strategies : a critical re-examination of sex differences in human and animal societies. *Philosophical Transactions of the Royal Society B*.
- Schacht, R., Rauch, K.L. & Borgerhoff Mulder, M. (2014) Too many men: the violence problem? *Trends in Ecology and Evolution*, **29**, 214–22.
- Schütz, C. & Schulze, C.H. (2015) Functional diversity of urban bird communities: Effects of landscape composition, green space area and vegetation cover. *Ecology and Evolution*, **5**, 5230–5239.
- Schwagmeyer, P.L. & Mock, D.W. (2008) Parental provisioning and offspring fitness: size matters. *Animal Behaviour*, **75**, 291–298.
- Şekercioğlu, Ç.H., Primack, R.B. & Wormworth, J. (2012) The effects of climate change on tropical birds. *Biological Conservation*, **148**, 1–18.
- Sepp, T., McGraw, K.J., Kaasik, A. & Giraudeau, M. (2018) A review of urban impacts on avian life-history evolution: Does city living lead to slower pace of life? *Global Change Biology*, **24**, 1452–1469.
- Seress, G., Bókony, V., Pipoly, I., Szép, T., Nagy, K. & Liker, A. (2012) Urbanization, nestling growth and reproductive success in a moderately declining house sparrow population. *Journal of Avian Biology*, **43**, 403–414.
- Seress, G., Hammer, T., Bókony, V., Vincze, E., Preiszner, B., Pipoly, I., Sinkovics, C., Evans, K.L. & Liker, A. (2018) Impact of urbanization on abundance and phenology of caterpillars and consequences for breeding in an insectivorous bird. *Ecological Applications*, **28**, 1143–1156.
- Seress, G. & Liker, A. (2015) Habitat urbanization and its effects on birds. *Acta Zoologica Academiae Scientiarum Hungaricae*, **61**, 373–408.

- Seress, G., Vincze, E., Pipoly, I., Hammer, T., Papp, S., Preiszner, B., Bókony, V. & Liker, A. (2017) Effects of capture and video-recording on the behavior and breeding success of Great Tits in urban and forest habitats. *Journal of Field Ornithology*, **88**, 299–312.
- Shaw, L., Chamberlain, D. & Evans, M. (2008) The House Sparrow (*Passer domesticus*) in urban areas: reviewing a possible link between post-decline distribution and human socioeconomic status. *Journal of Ornithology*, **149**, 293–299.
- Shultz, S., Bradbury, R.B., L Evans, K., Gregory, R.D. & Blackburn, T.M. (2005) Brain size and resource specialization predict long-term population trends in British birds. *Proceedings. Biological sciences / The Royal Society*, **272**, 2305–11.
- Silk, J.B. & Brown, G.R. (2008) Local resource competition and local resource enhancement shape primate birth sex ratios. *Proceedings of The Royal Society B*, **275**, 1761–5.
- Smith, P. a., Gilchrist, H.G., Forbes, M.R., Martin, J.-L. & Allard, K. (2010) Inter-annual variation in the breeding chronology of arctic shorebirds: effects of weather, snow melt and predators. *Journal of Avian Biology*, **41**, 292–304.
- Smith, H., Kallander, H. & Nilsson, J.-A. (1989) The trade-off between offspring number and quality in the great tit *Parus major*. *The Journal of Animal Ecology*, **58**, 383–401.
- Solow, A.R. (2017) On detecting ecological impacts of extreme climate events and why it matters. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **372**, 8–11.
- Stauss, M., Segelbacher, G., Tomiuk, J. & Bachmann, L. (2005) Sex Ratio of *Parus major* and *P. caeruleus* Broods Depends on Parental Condition and Habitat Quality. *Oikos*, **109**, 367–373.
- Strausberger, B.M. (1998) Temperature ,egg mass, and incubation time: a comparison of brown-headed cowbirds and red-winged blackbirds. *The Auk*, **115**, 843–850.
- Svensson, L. (1992) *Identification Guide to European Passerines*.
- Szász, E., Garamszegi, L.Z., Hegyi, G., Szöllösi, E., Markó, G., Török, J. & Rosivall, B. (2014) Aggressive behavior of the male parent predicts brood sex ratio in a songbird. *Naturwissenschaften*, **101**, 653–660.
- Szász, E., Garamszegi, L.Z. & Rosivall, B. (2019) What is behind the variation in mate quality dependent sex ratio adjustment? A meta analysis. *Oikos*, **128**, 1–12.
- Szász, E., Kiss, D. & Rosivall, B. (2012) Sex ratio adjustment in birds. *Ornis Hungarica*, **20**, 26–36.
- Székely, T., Liker, A., Freckleton, R.P., Fichtel, C. & Kappeler, P.M. (2014a) Sex-biased survival predicts adult sex ratio variation in wild birds. *Proceedings of The Royal Society B*, **281**.
- Székely, T., Weissing, F.J. & Komdeur, J. (2014b) Adult sex ratio variation: Implications for breeding system evolution. *Journal of Evolutionary Biology*, **27**, 1500–1512.
- Szép, T., Nagy, K., Nagy, Z. & Halmos, G. (2012) Population trends of common breeding and wintering birds in Hungary, decline of long-distance migrant and farmland birds during 1999–2012. *Ornis Hungarica*, **20**, 13–63.
- Tan, J., Zheng, Y., Tang, X., Guo, C., Li, L., Song, G., Zhen, X., Yuan, D., Kalkstein, A.J., Li, F. & Chen, H. (2010) The urban heat island and its impact on heat waves and human health in Shanghai. *International Journal of Biometeorology*, **54**, 75–84.
- Tanner, E.P., Elmore, R.D., Fuhlendorf, S.D., Davis, C.A., Dahlgren, D.K. & Orange, J.P. (2017) Extreme climatic events constrain space use and survival of a ground-nesting bird. *Global Change Biology*,

23, 1832–1846.

- Taylor, L.R. (1963) Analysis of the Effect of Temperature on Insects in Flight. *Journal of Animal Ecology*, **32**, 99–117.
- Tinbergen, J.M. & Boerlijst, M.C. (1990) Nestling Weight and Survival in Individual Great Tits (*Parus major*). *The Journal of Animal Ecology*, **59**, 1113.
- Torres, R. & Drummond, H. (1999) Variably male-biased sex ratio in a marine bird with females larger than males. *Oecologia*, **161**, 447–8.
- Trivers, R.L. (1972) Parental Investment and Sexual Selection. *Sexual Selection and the Descent of Man* p. Aldine, Chicago.
- Trivers, R.L. & Willard, D.E. (1973) Natural selection of parental ability to vary the sex ratio of offspring. *Science*, **179**, 90–92.
- Tschirren, B., Fitze, P.S. & Richner, H. (2003) Sexual dimorphism in susceptibility to parasites and cell-mediated immunity in great tit nestlings. *Journal of Animal Ecology*, **72**, 839–845.
- United Nations, Affairs, and Divisions (2014) *World Urbanization Prospects: The 2014 Revision, Highlights (ST/ESA/SER.A/352)*.
- Urban, M.C. (2015) Accelerating extinction risk from climate change. *Science*, **348**.
- Vangestel, C., Mergeay, J., Dawson, D.A., Vandomme, V. & Lens, L. (2011) Spatial heterogeneity in genetic relatedness among house sparrows along an urban-rural gradient as revealed by individual-based analysis. *Molecular Ecology*, **20**, 4643–4653.
- Végvári, Z., Bókony, V., Barta, Z. & Kovács, G. (2010) Life history predicts advancement of avian spring migration in response to climate change. *Global Change Biology*, **16**, 1–11.
- Végvári, Z., Katona, G., Vági, B., Freckleton, R.P., Gaillard, J.M., Székely, T. & Liker, A. (2018) Sex-biased breeding dispersal is predicted by social environment in birds. *Ecology and Evolution*, **8**, 6483–6491.
- Vincze, E., Pipoly, I., Seress, G., Preiszner, B., Papp, S., Németh, B., Liker, A. & Bókony, V. (2019) Great tits take greater risk toward humans and sparrowhawks in urban habitats than in forests. *Ethology*, **125**, 686–701.
- Vincze, O., Székely, T., Küpper, C., Alrashidi, M., Amat, J. a, Ticó, A.A., Burgas, D., Burke, T., Cavitt, J., Figuerola, J., Shobrak, M., Montalvo, T. & Kosztolányi, A. (2013) Local environment but not genetic differentiation influences biparental care in ten plover populations. *PLoS ONE*, **8**, e60998.
- Ward, K., Lauf, S., Kleinschmit, B. & Endlicher, W. (2016) Heat waves and urban heat islands in Europe: A review of relevant drivers. *Science of the Total Environment*, **569–570**, 527–539.
- Weatherhead, P.J. (2005) Effects of climate variation on timing of nesting, reproductive success, and offspring sex ratios of red-winged blackbirds. *Oecologia*, **144**, 168–75.
- Weathers, W.W. (1972) Physiological thermoregulation in heat-stressed birds: consequences of body sizes. *Physiological Zoology*, **54**, 345–361.
- Weeks, B.C., Willard, D.E., Ellis, A.A., Witynski, M.L. & Winger, B.M. (2019) Shared morphological consequences of global warming in North American migratory birds. *Ecology Letters*, 10.1111/ele.13434.
- Werren, J.H. & Beukeboom, L.W. (1998) Sex determination, sex ratios, and genetic conflict. *Annual Review of Ecology, Evolution, and Systematics*, **29**, 233–261.

- West, S. (2009) *Sex Allocation*, Princeton University Press.
- West, S.A., Herre, E. & Sheldon, B.C. (2000) The benefits of allocating sex. *Science*, **290**, 288–290.
- Westneat, D.F., Stewart, I.R.K., Woeste, E.H., Gipson, J., Abdulkadir, L. & Poston, J.P. (2002) Patterns of sex ratio variation in House Sparrows. *The Condor*, **104**, 598–609.
- Whitehouse, M.J., Harrison, N.M., Mackenzie, J. & Hinsley, S.A. (2013) Preferred Habitat of Breeding Birds May Be Compromised by Climate Change: Unexpected Effects of an Exceptionally Cold, Wet Spring. *PLoS ONE*, **8**, 1–13.
- Whitfield, M.C., Smit, B., Mckechnie, A.E. & Wolf, B.O. (2015) Avian thermoregulation in the heat : scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *The Journal of Experimental Biology*, **218**, 1705–1714.
- Wilson, E.O. (1975) *Sociobiology: The New Synthesis*. Harvard University Press, Cambridge.
- Winkler, D.W., Luo, M.K. & Rakhimberdiev, E. (2013) Temperature effects on food supply and chick mortality in tree swallows (*Tachycineta bicolor*). *Oecologia*, **173**, 129–38.
- Yamaguchi, N., Kawano, K.K., Eguchi, K. & Yahara, T. (2004) Facultative sex ratio adjustment in response to male tarsus length in the Varied Tit *Parus varius*. *Ibis*, **146**, 108–113.
- Yom-tov, Y., Yom-tov, S., Wright, J., Thorne, C.J.R. & Feu, R. Du. (2006) Recent changes in body weight and wing length among some British passerine birds. *Oikos*, 91–101.
- York, A. & Oberhauser, K. (2002) Effects of duration and timing of heat stress on Monarch butterfly (*Danaus plexippus*) (Lepidoptera : Nymphalidae) development. *Journal of the Kansas Entomological Society*, **75**, 290–298.
- Zahn, R.A., Morton, S.R., Jones, K.R. & Burley, N.T. (1995) The Timing of Breeding by Zebra Finches in Relation to Rainfall in Central Australia. *Emu*, **95**, 208–222.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer Science + Business Media, New York.

14. Appendices

A/1. Supplementary information for chapter 3

Table A/1.1. Annual values of reproductive and meteorological parameters in the study population between April-August

	2005	2006	2007	2008	2009	2010	Total
total number of eggs	248	296	219	277	265	122	1427
total number of hatched nestlings	202	227	157	187	189	103	1065
total number of fledged nestlings	138	152	129	125	134	58	736
hatchlings (nr.) / eggs (nr.)	0.815	0.767	0.717	0.675	0.713	0.844	0.746
fledglings (nr.) / hatchlings (nr.)	0.683	0.670	0.822	0.668	0.709	0.563	0.691
fledglings (nr.) / eggs (nr.)	0.556	0.514	0.589	0.451	0.506	0.475	0.516
total number of clutches	62	69	46	59	56	25	317
total number of hatched clutches	52	60	40	50	50	25	277
total number of fledged clutches	46	50	38	40	40	17	231
hatched / total clutches	0.839	0.870	0.870	0.847	0.893	1.000	0.874
fledged / hatched clutches	0.885	0.833	0.950	0.800	0.800	0.680	0.834
fledged / total clutches	0.742	0.725	0.826	0.678	0.714	0.680	0.729
mean body mass (g)	23.53	20.84	21.92	19.26	20.76	19.56	21.14
mean tarsus length (mm)	18.48	17.53	17.51	16.83	17.04	17.01	17.4
total number of known sexes	89	97	99	-	-	-	285
total number of males	40	48	52	-	-	-	140
total number of females	49	49	47	-	-	-	145
sex ratio (males/total)	0.449	0.495	0.525	-	-	-	0.491
clutches with known sex ratio	21	21	25	-	-	-	67
mean daily average temperature (°C)	16.55	18.02	19.18	16.37	18.82	17.81	17.87
total number of hot days	21	66	44	24	14	21	190
total number of cold days	103	76	10	54	42	40	325
total number of dry days	168	227	325	195	174	76	1165
total precipitation (mm)	667.4	409.5	376.0	244.5	290.0	524.7	2512.1
total number of rainy days	197	276	110	198	196	115	1092
total number of heavy rain days	48	66	16	66	27	21	244

Table A/1.2: Models with hatching success as dependent variable; $\Delta AIC < 2$ models are highlighted in bold (n=262 breeding attempts)

Variables in the models	AIC value	ΔAIC	Akaike weight
Date, I, T, P, H, C, CxI	1342.93	0.00	0.21
Date, I, T, P, H, C	1343.46	0.53	0.16
Date, I, T, P, H, C, R, CxI	1344.15	1.22	0.11
Date, I, T, P, H, C, R	1344.45	1.51	0.10
Date, I, T, P, H, C, D, CxI	1344.87	1.93	0.08
Date, I, T, P, H, C, D	1345.28	2.35	0.06
Date, I, T, P, H, C, D, R, CxI	1346.13	3.20	0.04
Date, I, T, P, H, C, D, R	1346.36	3.43	0.04
Date, I, T, P, H	1346.66	3.73	0.03
Date, I, T, P, C	1347.22	4.28	0.02
Date, I, T, P, C, CxI	1347.30	4.37	0.02
Date, I, T, P, C, R	1348.09	5.16	0.02
Date, I, T, P, H, R	1348.25	5.32	0.01
Date, I, T, P, C, R, CxI	1348.39	5.46	0.01
Date, I, T, P, H, D	1348.61	5.68	0.01
Date, I, T, P, C, D	1348.77	5.84	0.01
Date, I, T, P, C, D, CxI	1349.02	6.09	0.01
Date, I, T, P, C, D, R	1349.81	6.88	0.01
Date, I	1350.08	7.15	0.01
Date, I, T, P, H, D, R	1350.14	7.20	0.01
Date, I, T, P, C, D, R, CxI	1350.22	7.29	0.01
Date, I, P	1351.15	8.22	<0.01
Date, I, T	1351.91	8.98	<0.01
Date, I, T, P	1352.55	9.62	<0.01
Date, I, T, P, R	1354.15	11.21	<0.01
Date, I, T, P, D	1354.54	11.61	<0.01
Date, I, T, P, D, R	1356.11	13.18	<0.01

Date=Date of hatching of the first chick

T=daily mean temperature

P=Total amount of precipitation

H=Number of hot days

C=Number of cold days

D=Number of dry days

R=Number of heavy rain days

I=Incubation time

CxI=Interaction between number of cold days and incubation time

Table A/1.3: Models with fledging success as dependent variable; $\Delta AIC < 2$ models are highlighted in bold (n=211 breeding attempts)

Variables in the models	AIC value	ΔAIC	Akaike weight
Date, A	1286.17	0.00	0.22
Date, A, T	1287.28	1.11	0.13
Date, A, P	1287.54	1.37	0.11
Date, A, T, P, D	1288.91	2.73	0.06
Date, A, T, P	1288.98	2.81	0.06
Date, A, T, P, D, R	1289.51	3.34	0.04
Date, A, T, P, H, D	1289.62	3.45	0.04
Date, A, T, P, H	1289.74	3.57	0.04
Date, A, T, P, R	1289.89	3.71	0.04
Date, A, T, P, H, C, D,	1290.09	3.91	0.03
Date, A, T, P, C, D	1290.10	3.93	0.03
Date, A, T, P, C, D, R	1290.17	4.00	0.03
Date, A, T, P, C	1290.23	4.06	0.03
Date, A, T, P, H, C	1290.29	4.12	0.03
Date, A, T, P, H, C, D, R	1290.49	4.32	0.03
Date, A, T, P, H, D, R	1290.55	4.38	0.03
Date, A, T, P, C, R	1290.69	4.52	0.02
Date, A, T, P, H, R	1290.93	4.76	0.02
Date, A, T, P, H, C, R	1291.05	4.88	0.02

Date=Date of hatching of the first chick
 T=daily mean temperature
 P=Total amount of precipitation
 H=Number of hot days
 C=Number of cold days
 D=Number of dry days
 R=Number of heavy rain days
 A=Age of nestlings at measuring day

Table A/1.4: Models with average nestling mass as dependent variable; $\Delta AIC < 2$ models are highlighted in bold (n=211 breeding attempts)

Variables in the models	AIC value	ΔAIC	Akaike weight
Date, A, B, T, P, H, D	1325.26	0.00	0.31
Date, A, B, T, P, D	1326.15	0.89	0.20
Date, A, B, T, P, H, D, R	1327.05	1.79	0.13
Date, A, B, T, P, H, C, D,	1327.25	1.99	0.11
Date, A, B, T, P, C, D	1327.82	2.56	0.09
Date, A, B, T, P, D, R	1328.10	2.84	0.07
Date, A, B, T, P, H, C, D, R	1329.01	3.75	0.05
Date, A, B, T, P, C, D, R	1329.70	4.44	0.03
Date, A, B, T	1333.44	8.18	0.01
Date, A, B, T, P, H	1334.39	9.13	0.00
Date, A, B, T, P	1335.43	10.17	0.00
Date, A, B, T, P, H, R	1336.36	11.10	0.00
Date, A, B, T, P, H, C	1336.38	11.12	0.00
Date, A, B, T, P, C	1337.12	11.86	0.00
Date, A, B, T, P, R	1337.42	12.16	0.00
Date, A, B, T, P, H, C, R	1338.35	13.09	0.00
Date, A, B, T, P, C, R	1339.12	13.86	0.00
Date, A, B	1346.76	21.50	0.00
Date, A, B, P	1347.17	21.91	0.00

Date=Date of hatching of the first chick

T=daily mean temperature

P=Total amount of precipitation

H=Number of hot days

C=Number of cold days

D=Number of dry days

R=Number of heavy rain days

A=Age of nestlings at measuring

B=Brood size

Table A/1.5: Models with average nestling tarsus length as dependent variable; $\Delta AIC < 2$ models are highlighted in bold (n=210 breeding attempts)

Variables in the models	AIC value	ΔAIC	Akaike weight
Date, A, B, T	1319.93	0.00	0.15
Date, A, B, T, P, H, C, D,	1320.43	0.50	0.12
Date, A, B, T, P, H, D	1320.61	0.69	0.11
Date, A, B, T, P, H, C	1320.72	0.80	0.10
Date, A, B, T, P, H	1320.95	1.02	0.09
Date, A, B, T, P, D	1321.48	1.55	0.07
Date, A, B, T, P	1321.91	1.98	0.06
Date, A, B, T, P, H, D, R	1322.20	2.27	0.05
Date, A, B, T, P, H, C, D, R	1322.28	2.35	0.05
Date, A, B, T, P, C, D	1322.58	2.65	0.04
Date, A, B, T, P, H, C, R	1322.67	2.74	0.04
Date, A, B, T, P, H, R	1322.71	2.78	0.04
Date, A, B, T, P, C	1322.99	3.07	0.03
Date, A, B, T, P, D, R	1323.31	3.39	0.03
Date, A, B, T, P, R	1323.85	3.92	0.02
Date, A, B, T, P, C, D, R	1324.53	4.60	0.01
Date, A, B, T, P, C, R	1324.99	5.07	0.01
Date, A, B	1328.05	8.13	0.00
Date, A, B, P	1328.91	8.98	0.00

Date=Date of hatching of the first chick

T=daily mean temperature

P=Total amount of precipitation

H=Number of hot days

C=Number of cold days

D=Number of dry days

R=Number of heavy rain days

A=Age of nestlings at measuring

B=Brood size

Table A/1.6: Model set for sex ratio at fledging in house sparrows; models with $\Delta AIC > 2$ are written in bold (n=61 nests)

Variables in the models	AIC value	ΔAIC	Akaike weight
Date	424.309	0	0.26
Date, T	426.133	1.824	0.10
Date, P	426.303	1.994	0.09
Date, T, P, D	426.883	2.574	0.07
Date, T, P, H, D	427.353	3.044	0.06
Date, T, P, D, R	427.637	3.328	0.05
Date, T, P, R	427.815	3.506	0.05
Date, T, P, H, D, R	427.988	3.679	0.04
Date, T, P	428.079	3.77	0.04
Date, T, P, H, R	428.136	3.827	0.04
Date, T, P, H	428.555	4.246	0.03
Date, T, P, C, D	428.655	4.346	0.03
Date, T, P, H, C, D,	428.725	4.416	0.03
Date, T, P, H, C, R	429.222	4.913	0.02
Date, T, P, H, C, D, R	429.326	5.017	0.02
Date, T, P, C, D, R	429.4	5.091	0.02
Date, T, P, C, R	429.425	5.116	0.02
Date, T, P, H, C	429.626	5.317	0.02
Date, T, P, C	429.661	5.352	0.02

Date=Date of hatching of the first chick

T=daily mean temperature

P=Total amount of precipitation

H=Number of hot days

C=Number of cold days

D=Number of dry days

R=Number of heavy rain days

A/2. Supplementary information for chapter 5

Table A/2.1. Number of broods and offspring used in different models. Full models include parents' tarsus length as covariate, whereas tarsus length was excluded from reduced models.

Sex ratio	Model	Broods	Offspring
First and second annual broods together			
Primary	Full	62	622
	Reduced	79	793
Fledgling	Full	105	943
	Reduced	126	1153
First annual broods only			
Primary	Full	46	498
	Reduced	59	642
Fledgling	Full	81	772
	Reduced	98	956

Table A/2.2. Primary sex ratio (n= 62 broods) and fledgling sex ratio (n= 105 broods) in relation to site, year, laying date and parents' tarsus length (first and second annual broods pooled). Estimates with SE were calculated from the parameter estimates of generalized linear mixed-effects models with binomial error distribution and “logit” link function, with brood ID nested in pair ID as random factors. Year parameters show the difference from 2012.

Model parameters	Estimate±SE	t	P
Primary sex ratio			
site: Veszprém city	2.21±3.881	0.570	0.5713
site: Balatonfüred (city)	1.96±0.318	0.491	0.4321
site: Vilma-puszta (forest)	2.00±0.264	0.504	0.4325
site: Szentgál (forest)	2.45±0.246	0.612	0.3328
year: 2013	0.13±0.338	0.390	0.7103
year: 2014	0.32±0.324	0.987	0.3795
Laying date (Julian day)	0.006±0.005	1.229	0.2865
Mother's tarsus length	-0.100±0.144	-0.701	0.4866
Father's tarsus length	-0.058±0.163	-0.359	0.7378
Fledgling sex ratio			
site: Veszprém city	-0.48±3.243	-0.148	0.8824
site: Balatonfüred (city)	-0.69±0.257	-0.209	0.8350
site: Vilma-puszta (forest)	-0.70±0.198	-0.209	0.8349
site: Szentgál (forest)	-0.56±0.190	-0.167	0.8681
year: 2013	0.120±0.234	0.513	0.6169
year: 2014	0.101±0.222	0.455	0.6564
Laying date (Julian day)	0.004±0.004	1.081	0.2994
Mother's tarsus length	-0.041±0.116	-0.352	0.7256
Father's tarsus length	0.045±0.128	0.352	0.7307

Table A/2.3. Primary and fledgling sex ratio of great tits in relation to study site, year, and laying date mean-centered for study site and year (first and second annual broods pooled). Effects are presented as analysis of deviance tables with type-2 sums of squares for the reduced generalized mixed-effects models; $n = 79$ and 126 for primary and fledgling sex ratios, respectively.

	χ^2	df	<i>P</i>
Primary sex ratio			
Sites	3.354	3	0.340
Years	0.270	2	0.874
Mean-centered laying date	1.583	1	0.208
Fledgling sex ratio			
Sites	0.994	3	0.803
Years	3.591	2	0.166
Mean-centered laying date	2.425	1	0.119

Table A/2.4. Primary sex ratio (n= 59 broods) and fledgling sex ratio (n= 98 broods) in relation to site, year, and laying date (first annual broods only). Estimates with SE were calculated from the parameter estimates of generalized linear mixed-effects models with binomial error distribution and “logit” link function, with brood ID nested in pair ID as random factors. Year parameters show the difference from 2012.

Model parameters	Estimate±SE	t	P
Primary sex ratio			
site: Veszprém (city)	-1.64±2.443	-0.672	0.5042
site: Balatonfüred (city)	-2.04±2.461	-0.830	0.4104
site: Vilma-puszta (forest)	-1.92±2.569	-0.749	0.4574
site: Szentgál (forest)	-1.72±2.548	-0.677	0.5014
year: 2013	-0.07±0.364	-0.180	0.8582
year: 2014	0.26±0.520	0.492	0.6249
Laying date (Julian day)	0.02±0.024	0.682	0.4982
Fledgling sex ratio			
site: Veszprém city	-1.75±1.380	-1.266	0.2087
site: Balatonfüred (city)	-2.01±1.433	-1.399	0.1650
site: Vilma-puszta (forest)	-2.043±1.449	-1.410	0.1619
site: Szentgál (forest)	-2.08±1.465	-1.421	0.1588
year: 2013	-0.06±0.259	-0.231	0.8387
year: 2014	0.225±0.325	0.693	0.5600
Laying date (Julian day)	0.020±0.014	1.414	0.2929

Table A/2.5. Urban-forest differences (linear contrasts) in sex ratio of first annual broods. OR refers to the odds ratio of an offspring being male instead of female at urban sites opposed to forest sites. Full models contain the parents' tarsus length as covariate.

Urban vs. forest	OR [95%CI]	df	t	<i>P</i>
Primary sex ratio				
Full model	0.95 [0.73;1.24]	37	-0.178	0.860
Reduced model	0.98 [0.78;1.24]	52	-0.079	0.937
Fledgling sex ratio				
Full model	1.17 [0.97;1.42]	72	0.859	0.393
Reduced model	1.20 [1.00;1.43]	90	1.055	0.294

Table A/2.6. Body size parameters of nestlings in relation to sex, site, year, and laying date (Julian day; first and second annual broods pooled). Parameter estimates with standard error (SE) are shown from linear mixed-effects models, with brood ID nested in pair ID and crossed with measurer ID as random factors. Site parameters show the differences from Veszprém, year parameters show the difference from 2012; the intercept refers to Veszprém 2012.

Model parameters	Estimate±SE	t	P
Wing length (mm)			
Intercept	47.11±1.951	24.149	<0.001
sex: male	0.47±0.18	2.546	0.011
site: Balatonfüred (city)	2.58±1.30	1.992	0.049
site: Vilma-puszta (forest)	2.27±0.87	2.603	0.002
site: Szentgál (forest)	2.56±0.83	3.098	0.011
year: 2013	0.44±1.11	0.397	0.692
year: 2014	0.98±1.08	0.910	0.365
Laying date (Julian day)	-0.004±0.017	-0.259	0.796
Tarsus length (mm)			
Intercept	19.03±0.327	58.224	<0.001
sex: male	0.47±0.030	15.509	<0.001
site: Balatonfüred (city)	0.27±0.210	1.307	0.194
site: Vilma-puszta (forest)	0.52±0.139	3.731	<0.001
site: Szentgál (forest)	0.61±0.134	4.548	<0.001
year: 2013	0.11±0.178	0.627	0.532
year: 2014	0.23±0.175	1.315	0.191
Laying date (Julian day)	-0.001±0.003	-0.214	0.831
Body mass (g)			
Intercept	14.83±0.898	16.512	<0.001
sex: male	0.58±0.059	9.856	<0.001
site: Balatonfüred (city)	1.15±0.589	1.959	0.053
site: Vilma-puszta (forest)	1.72±0.389	4.410	<0.001
site: Szentgál (forest)	2.61±0.370	7.064	<0.001
year: 2013	0.04±0.506	0.087	0.931
year: 2014	0.47±0.483	0.972	0.333
Laying date (Julian day)	-0.001±0.008	-0.082	0.935

A/3. Supplementary information for chapter 7**Table A/3.1. Detailed analyses of the effect of sex-determination system on the adult sex ratio.**

These are extensions of Table 7.1 in the text showing details of the phylogenetically uncorrected (t-tests) and phylogenetically corrected analyses (PGLS, Pagel 1998 and PDM, Pagel 1994). Birds and mammals were not tested with phylogenetic control because there is no variation in the type of sex-determination system within birds and mammals. In the reduced analysis (marked by †) snakes, birds and mammals were each included as a single datum with mean species values.

	Species level		Phylogenetically corrected			
	t-test		PDM		PGLS	
	t-value	p-value (n)	LR	p-value (n)	t-value	p-value (n)
Amphibians (XY vs. ZW)	3.039	0.008 (39)	10.5	0.033 (39)	3.418	0.002 (39)
Reptiles (XY vs. ZW)	6.018	< 0.001 (67)	11.3	0.023 (67)	5.996	< 0.001 (67)
Mammals (XY) vs. birds (ZW)	8.982	< 0.001 (238)	not tested		not tested	
Tetrapods, all species (XY vs. ZW)	9.790	< 0.001 (344)	53.6	< 0.001 (344)	5.313	< 0.001 (344)
Tetrapods, reduced † (XY vs. ZW)	4.801	< 0.001 (87)	17.9	0.001 (87)	5.072	< 0.001 (87)

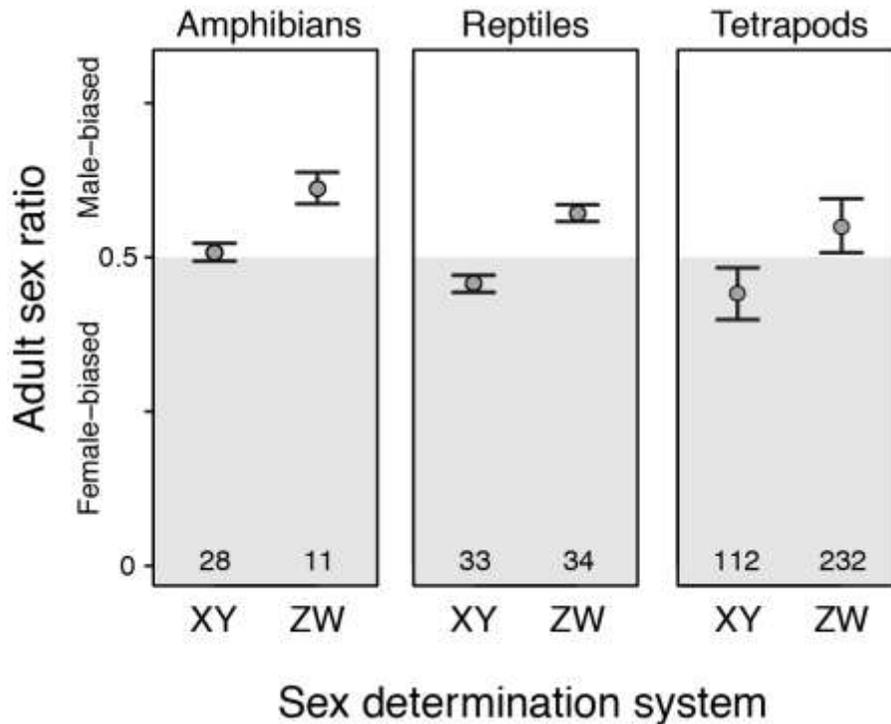
Table A/3.2. Phylogenetically controlled analyses of the relationship between adult sex ratio and genetic sex-determination system using different branch length assumptions.

These are results of phylogenetic generalized least squares models (PGLS) as implemented in the R package ‘caper’ (Orme *et al.* 2013), showing parameter estimates (b) as the difference in ASR (ZW – XY), the proportion of interspecific variance (R^2) in ASR explained by the sex-determination system (female-heterogametic, ZW or male-heterogametic, XY), calculated by PGLS; and the degree of phylogenetic dependence (λ). The models assume gradual branch lengths calculated either by Nee’s or by Pagel’s method, or unit branch lengths (Maddison & Maddison 2011).

Taxa	Branch lengths	b ± SE	t	p	R^2	λ
Amphibians (n = 39)	Nee’s	0.101 ± 0.030	3.418	0.002	0.240	0.000
	Pagel’s	0.101 ± 0.030	3.418	0.002	0.240	0.000
	Unit branch lengths	0.076 ± 0.027	2.821	0.008	0.177	0.000
Reptiles (n = 67)	Nee’s	0.114 ± 0.019	5.996	< 0.001	0.356	0.000
	Pagel’s	0.114 ± 0.019	5.968	< 0.001	0.354	0.000
	Unit branch lengths	0.114 ± 0.020	5.702	< 0.001	0.333	0.000
Tetrapods (n = 344)	Nee’s	0.109 ± 0.020	5.313	< 0.001	0.076	0.409
	Pagel’s	0.106 ± 0.021	4.998	< 0.001	0.068	0.332
	Unit branch lengths	0.093 ± 0.020	4.581	< 0.001	0.058	0.469

Figure A/3.1. Phylogenetically corrected mean and standard error of adult sex ratio in clades with different sex-determination systems.

Parameter estimates for means and the associated standard errors were calculated by phylogenetic generalized least squares models (PGLS)¹⁸ presented in Table A/2.2 (with branch lengths estimated by Nee's method⁵⁵).

**Other analyses to validate and strengthen our results**

On the collected ASR data, we tested whether ASR estimates differ between sampling (hand-capture, trap, other) and marking (mark-recapture, sacrifice) methods, and we found no such differences (linear mixed-effects model with species as random factor, sampling: $F_{(3, 105)} = 0.50$, $P = 0.683$; marking: $F_{(2, 105)} = 2.18$, $P = 0.118$; $n = 234$ records). Weighted and non-weighted mean ASRs were highly correlated (amphibians: Pearson's $r = 0.973$, $P < 0.001$, $n = 35$ species; reptiles: $r = 0.995$, $P < 0.001$, $n = 60$ species); we used non-weighted averages because not all studies reported sample size.

We used reptile species where GSD was known at least for family-level. Our result is not changed qualitatively by restricting our analyses to those reptile species for which GSD is known at species level, i.e. when species for which we assumed GSD based on other species in the family were excluded (difference between XY and ZW reptile species, phylogenetic generalized least squares model (PGLS: $b \pm SE = 0.11 \pm 0.02$; $t = 4.70$, $P < 0.001$, $n = 26$; $R^2 = 0.479$).

The results of the multivariate PGLS model of tetrapods presented in Table 7.2 remain qualitatively the same when wing length dimorphism (data available for $n = 153$ species) is used for birds instead of body mass dimorphism (effect of sex determination: $b \pm SE = -0.10 \pm 0.02$, $t = 4.97$, $P < 0.001$; body size: $b \pm SE = 0 \pm 0$, $t = 0.06$, $P = 0.949$; latitude: $b \pm SE = 0 \pm 0$, $t = 0.223$, $P = 0.823$; size dimorphism: $b \pm SE = -0.52 \pm 0.12$, $t = 4.33$, $P < 0.001$; $n = 248$ species).

The difference between XY and ZW systems for tetrapods in the bivariate model is not sensitive to the inclusion of large clades with uniform sex-determination systems (snakes and birds are all ZW, mammals are all XY) since it remains unchanged when each of these clades is reduced to a single datum of its mean ASR (PGLS: $b \pm SE = 0.10 \pm 0.02$, $t = 5.07$, $P < 0.001$, $R^2 = 0.232$, $n = 87$). Furthermore, our result is also robust to between-species differences in sample size: when we added $\log(\text{nr. individuals})$ to the previous model, the effect of sex determination remained significant ($b \pm SE = 0.15 \pm 0.07$, $t = 2.08$, $P = 0.041$) while sample size had no significant effect on ASR ($b \pm SE = 0 \pm 0.01$, $t = 0.35$, $P = 0.72$, $n = 78$). Furthermore, sample size was not a significant predictor of ASR when we added it as a fourth confounding variable in the full PGLS model ($b \pm SE = 0 \pm 0.01$, $t = 1.16$, $P = 0.250$, $n = 78$), and the effect of other predictors remained qualitatively the same as in Table 7.2. Finally, the results do not change when we only used the most reliable ASR data (based on mark-recapture or sacrifice methods): sex-determination system is significantly related to ASR in amphibians, reptiles tetrapods (PGLS results, amphibians: $b \pm SE = 0.09 \pm 0.03$, $t = 3.07$, $P = 0.004$, $n = 35$ species; reptiles: $b \pm SE = 0.11 \pm 0.03$, $t = 3.974$, $P < 0.001$, $n = 22$; tetrapods with snakes, birds and mammals included as single data points: $b \pm SE = 0.10 \pm 0.02$, $t = 4.23$, $P < 0.001$, $n = 55$).

Analyses about the possible confounding effects of the lifespan of individuals and sex-specific dispersal distances: First, longer average lifespan may lead to exaggeration of ASR bias. However, in species with available data (Healy *et al.* 2014), lifespan is unrelated to ASR (PGLS, birds: $b \pm SE = 0 \pm 0$, $t = 0.196$, $P = 0.845$, $n = 71$ species; mammals: $b \pm SE = 0 \pm 0$, $t = 0.751$, $P = 0.457$, $n = 35$ species) and also to the absolute deviation of ASR from 0.5 (i.e. when assuming that longer lifespan can exaggerate ASR bias in either direction; birds: $b \pm SE = 0 \pm 0$, $t = 1.543$, $P = 0.127$, $n = 71$ species; mammals: $b \pm SE = 0 \pm 0$, $t = 0.180$, $P = 0.858$, $n = 35$ species). Second, sex-specific dispersal can bias ASR due to the higher mortality in the sex with longer dispersal distances. However, we found no evidence of a relationship of sex bias in dispersal either with GSD in reptiles, or with ASR in birds (Appendix A/3.1). For these reasons, as well as because data on lifespan and/or sex-specific dispersal are not available for most species in our ASR data set, we did not include these variables in the main multi-predictor models.

A/3.1: Analyses of sex-biased dispersal

1. The relationship between sex-biased dispersal and sex determination in reptiles

A possible behavioural reason for biased adult sex ratio (ASR) could be sex-specific dispersal, because members of the sex with longer dispersal distance may suffer higher rate of mortality during their movements. Thus, if sex-biased dispersal is associated with the type of genetic sex determination (GSD), this could potentially generate a spurious relationship between ASR and GSD.

To test for a relationship between sex bias in dispersal and GSD, we conducted a preliminary literature search of published studies on sex differences in dispersal in amphibians and reptiles with known GSD. We found sufficient dispersal data for both XY and ZW species only for reptiles (28 species, data presented in Table SI A/3.1/1). In these species we determined the sex dispersing farther on the basis of explicit statements of the source papers; we treat dispersal unbiased or variable when no statistical differences were found between the sexes or the direction of bias was inconsistent between samples, respectively. Out of 10 species with XY-type sex determination, 80% has male-biased dispersal and 20% shows no or variable sex bias in dispersal. Similarly, out of 18 species with ZW-type sex determination, 67% has male-biased dispersal, whereas 5% and 28% has female-biased and unbiased or variable dispersal, respectively. In this sample GSD is not associated with sex bias in dispersal (categorized as male-biased versus not male-biased; Pagel's Discrete Method¹⁹, LR = 1.64, $P = 0.801$). This finding that reptiles tend to have male-biased dispersal is consistent with the conclusions of recent studies on sex-specific dispersal in snakes⁵⁸ and in lizards²². Thus, available data suggest that sex bias in dispersal and GSD are not associated in reptiles.

Table SI A/3.1/1 Data on sex-biased dispersal in 28 reptile species, and their sources.

Family	Species	GSD*	Sex bias in dispersal	Reference for dispersal data
Iguanidae	<i>Amblyrhynchus cristatus</i>	XY	male	59
Iguanidae	<i>Anolis limifrons</i>	XY	male	60
Iguanidae	<i>Anolis oculatus</i>	XY	male	61
Iguanidae	<i>Anolis roquet</i>	XY	male	62
Iguanidae	<i>Anolis sagrei</i>	XY	male	63
Iguanidae	<i>Crotaphytus collaris</i>	XY	no or variable	64
Iguanidae	<i>Ctenosaura pectinata</i>	XY	male	65
Iguanidae	<i>Sceloporus occidentalis</i>	XY	male	66
Iguanidae	<i>Uta stansburiana</i>	XY	male	67
Scincidae	<i>Egernia whitii</i>	XY	no or variable	68
Acrochordidae	<i>Acrochordus arafurae</i>	ZW	female	69
Agamidae	<i>Phrynocephalus przewalskii</i>	ZW	male	70
Agamidae	<i>Phrynocephalus vlangalii</i>	ZW	male	71
Boidae	<i>Boa constrictor</i>	ZW	male	72
Colubridae	<i>Cerberus schneiderii</i>	ZW	no or variable	73
Colubridae	<i>Coronella austriaca</i>	ZW	male	74
Colubridae	<i>Stegonotus cucullatus</i>	ZW	male	75
Colubridae	<i>Thamnophis atratus</i>	ZW	male	76
Colubridae	<i>Thermophis baileyi</i>	ZW	male	77
Elapidae	<i>Aipysurus laevis</i>	ZW	no or variable	78
Elapidae	<i>Cryptophis nigrescens</i>	ZW	male	79
Elapidae	<i>Hoplocephalus bungaroides</i>	ZW	no or variable	80, 81
Elapidae	<i>Laticauda laticaudata</i>	ZW	no or variable	82
Elapidae	<i>Laticauda saintgironsi</i>	ZW	no or variable	82
Gekkonidae	<i>Gehyra variegata</i>	ZW	male	83
Lacertidae	<i>Lacerta agilis</i>	ZW	male	84
Lacertidae	<i>Lacerta vivipara</i>	ZW	male	85, 86
Lacertidae	<i>Podarcis sicula</i>	ZW	male	87

*Genetic sex-determination system (source: see Supplementary Data^{34, 36, 37})

2. Sex-biased dispersal and ASR in birds

We directly tested whether ASR bias is related to sex-biased dispersal using birds where published data⁸⁸ on sex-specific dispersal distances (in meters) were available for 21 species from our ASR data set (Table SI A/3.1/2). For this analysis we calculated sex bias in dispersal distance as $\log_{10}(\text{female dispersal distance} / \text{male dispersal distance})$. We used ASR as response variable and sex bias in dispersal distance as predictor variable in the phylogenetic model. This analysis shows that sex bias in dispersal distance is not significantly associated with ASR in birds (phylogenetic generalized least squares [PGLS]¹⁸: $b \pm \text{s.e.m.} = 0.083 \pm 0.06$, $t = 1.42$, $P = 0.171$).

Table SI A/3.1/2 Data on sex bias in dispersal distance in birds.

Family	Species	ASR	Male dispersal distance (m)	Female dispersal distance (m)	Sex bias in dispersal distance
Accipitridae	Accipiter gentilis	0.481	27741	15588	-0.250
Accipitridae	Accipiter nisus	0.47	9660	18340	0.278
Accipitridae	Circus cyaneus	0.338	6300	5680	-0.045
Anatidae	Branta canadensis	0.501	15200	3100	-0.690
Anatidae	Cygnus olor	0.584	12859	7754	-0.220
Cinclidae	Cinclus cinclus	0.49	3090	6450	0.320
Corvidae	Aphelocoma coerulescens	0.537	387	1165	0.479
Corvidae	Pica pica	0.58	358	465	0.114
Emberizidae	Melospiza melodia	0.505	110	127	0.062
Emberizidae	Passerculus sandwichensis	0.491	262	309	0.072
Emberizidae	Zonotrichia leucophrys	0.5	555	614	0.044
Falconidae	Falco peregrinus	0.58	58000	83000	0.156
Hirundinidae	Hirundo rustica	0.585	6375	8125	0.105
Maluridae	Malurus splendens	0.576	100	200	0.301
Muscicapidae	Saxicola rubetra	0.52	500	500	0.000
Phasianidae	Centrocercus urophasianus	0.278	7400	8800	0.075
Phasianidae	Lagopus lagopus	0.525	1000	2900	0.462
Phasianidae	Lagopus leucura	0.579	1250	4000	0.505
Phasianidae	Tetrao tetrix	0.61	1500	8000	0.727
Picidae	Melanerpes formicivorus	0.6	220	530	0.382
Troglodytidae	Troglodytes aedon	0.524	608	674	0.045

3. The effect of sex-determination system on ASR in models that control for sex-biased dispersal

We used the subset of species that overlaps between our ASR data set and the available dispersal data sets (see SI tables in A/3 above), plus six amphibians we found by searching the literature (see A/3 section 1. above) and three mammalian species from Ref. 88 to investigate whether the relationship between sex-determination system and ASR remains significant when the influence of sex-biased dispersal is taken into account in multi-predictor models. In these analyses sex-biased dispersal was included as a binary trait with 'male-biased' and 'not male-biased' states, the latter including species with female-biased, unbiased and variable dispersal. All data and the sources of sex-biased dispersal are shown in Table SI A/3.1/3.

First, we constructed two conservative models that include the GSD-uniform snakes, birds, and mammals as a single datum each (corresponding to models in the main analyses, see lines 490-506 in main text and Table A/3.1), because our data set is strongly biased toward birds (21 of 40 species). In these models dispersal was included for snakes as male-biased (see A/3 section 1, above), for birds as not male-biased, and for mammals as male-biased. We found that ASRs are significantly more male-biased in species with ZW sex-determination system than in species with XY sex-determination system when sex-bias in dispersal is controlled for (Model 1 in Table SI A/3.1/4). This result is not sensitive to the dispersal categorisation of the snakes since the significant effect of sex-determination system on ASR remains when snakes are included as not male-biased instead of male-biased (effect of GSD in PGLS: $b (\pm \text{s.e.m.}) = 0.10 (\pm 0.04)$, $t = 2.828$, $P = 0.013$, $n = 17$). The effect of sex-determination system on ASR also tends to be significant when all confounding variables (body size, sexual size dimorphism, breeding latitude and sex-biased dispersal) were included in the model (Model 2 in Table SI A/3.1/4). Note that sample size is small relative to the number of predictors in this latter model. Finally, GSD predicts the ASR significantly even when we use all species (including snakes, birds and mammals) as individual data points (Model 3 in Table SI A/3.1/4).

These results, together with those presented in sections 1 & 2 above, consistently show that the relationship between sex determination and ASR is not confounded by the influence of sex-biased dispersal.

Table SI A/3.1/3 ASR and sex-biased dispersal in tetrapods, and data sources.

Taxon	Species	ASR	GSD	Sex-bias in dispersal	Dispersal reference	Body size	Sexual size dimorphism	Latitude
Amphibians	<i>Bufo bufo</i>	0.75	ZW	not male	⁸⁹	66.16	-0.081	51.64
Amphibians	<i>Hyla arborea</i>	0.55	XY	male	⁹⁰	43.90	-0.013	46.53
Amphibians	<i>Rana catesbeiana</i>	0.47	XY	not male	⁹¹	131.96	-0.032	44.02
Amphibians	<i>Rana lessonae</i>	0.51	XY	not male	⁹²	59.65	0.033	52.00
Amphibians	<i>Rana ridibunda</i>	0.44	XY	male	⁹²	70.06	-0.013	49.58
Amphibians	<i>Triturus alpestris</i>	0.49	XY	male	⁹³	50.83	-0.066	48.20
Reptiles	<i>Acrochordus arafurae</i>	0.56	ZW	not male	⁷⁷	870.00	-0.119	-12.61
Reptiles	<i>Anolis sagrei</i>	0.44	XY	male	⁶³	42.95	0.086	25.59
Reptiles	<i>Boa constrictor</i>	0.53	ZW	male	⁷²	1925.00	-0.056	-31.83
Reptiles	<i>Cerberus schneiderii</i>	0.51	ZW	not male	⁷³	407.55	0.009	1.45
Reptiles	<i>Egernia whitii</i>	0.49	XY	not male	⁶⁸	85.15	-0.018	-35.88
Reptiles	<i>Gehyra variegata</i>	0.46	ZW	male	⁸³	51.40	0.009	-31.63
Reptiles	<i>Lacerta agilis</i>	0.48	ZW	male	⁸⁴	71.60	-0.027	50.90
Reptiles	<i>Lacerta vivipara</i>	0.45	ZW	male	^{85, 86}	51.16	-0.042	48.16
Reptiles	<i>Podarcis sicula</i>	0.57	ZW	male	⁸⁷	64.20	0.037	45.51
Reptiles	<i>Uta stansburiana</i>	0.44	XY	male	⁶⁷	47.00	0.037	36.63
Birds	<i>Accipiter gentilis</i>	0.48	ZW	male	⁸⁸	---	-0.204	43.74
Birds	<i>Accipiter nisus</i>	0.47	ZW	not male	⁸⁸	---	-0.286	48.71
Birds	<i>Aphelocoma coerulescens</i>	0.54	ZW	not male	⁸⁸	96.75	0.025	28.00
Birds	<i>Branta canadensis</i>	0.5	ZW	male	⁸⁸	631.93	0.061	51.91
Birds	<i>Centrocercus urophasianus</i>	0.28	ZW	not male	⁸⁸	---	0.275	43.89
Birds	<i>Cinclus cinclus</i>	0.49	ZW	not male	⁸⁸	113.00	0.063	48.22
Birds	<i>Circus cyaneus</i>	0.34	ZW	male	⁸⁸	229.35	-0.183	50.08
Birds	<i>Cygnus olor</i>	0.58	ZW	male	⁸⁸	1181.90	0.086	49.75
Birds	<i>Falco peregrinus</i>	0.58	ZW	not male	⁸⁸	269.75	-0.174	10.88
Birds	<i>Hirundo rustica</i>	0.59	ZW	not male	⁸⁸	98.40	-0.014	16.80
Birds	<i>Lagopus lagopus</i>	0.53	ZW	not male	⁸⁸	262.05	0.058	61.16
Birds	<i>Lagopus leucura</i>	0.58	ZW	not male	⁸⁸	---	0.010	51.39
Birds	<i>Malurus splendens</i>	0.58	ZW	not male	⁸⁸	50.90	0.043	-27.70
Birds	<i>Melanerpes formicivorus</i>	0.6	ZW	not male	⁸⁸	119.50	0.02	23.58
Birds	<i>Melospiza melodia</i>	0.51	ZW	not male	⁸⁸	57.17	0.029	40.78
Birds	<i>Passerculus sandwichensis</i>	0.49	ZW	not male	⁸⁸	73.07	0.000	45.11
Birds	<i>Pica pica</i>	0.58	ZW	not male	⁸⁸	163.20	0.030	43.08
Birds	<i>Saxicola rubetra</i>	0.52	ZW	not male	⁸⁸	71.20	-0.005	54.13
Birds	<i>Tetrao tetrix</i>	0.61	ZW	not male	⁸⁸	308.00	0.116	55.06

Birds	Troglodytes aedon Zonotrichia	0.52	ZW	not male	88	66.65	-0.042	1.97
Birds	leucophrys	0.54	ZW	not male	88	---	0.054	52.01
Mammals	Helogale parvula	0.48	XY	not male	88	---	---	-7.04
Mammals	Lycaon pictus	0.59	XY	male	88	---	---	-2.74
Mammals	Ursus americanus	0.33	XY	male	88	---	---	47.57

Table SI A/3.1/4 The relationships between adult sex ratio, sex-determination system, dispersal, and other confounding factors in phylogenetically corrected multi-predictor analyses.

	Model 1 (n = 17)			Model 2* (n = 17)			Model 3 (n = 32 species)		
	<i>b</i> (± s.e.m.)	<i>t</i>	<i>P</i>	<i>b</i> (± s.e.m.)	<i>t</i>	<i>P</i>	<i>b</i> (± s.e.m.)	<i>t</i>	<i>P</i>
Sex-determination system	0.115 (± 0.04)	3.17	0.007	0.072 (± 0.04)	1.93	0.080	0.092 (± 0.04)	2.52	0.018
Sex bias in dispersal	0.063 (± 0.03)	2.23	0.043	0.060 (± 0.04)	1.52	0.157	0.068 (± 0.02)	2.87	0.008
Body size	---	---	---	0 (± 0)	0.52	0.614	0 (± 0)	1.17	0.251
Breeding latitude	---	---	---	0 (± 0)	0.11	0.913	0 (± 0)	0.66	0.516
Sexual size dimorphism	---	---	---	-0.627 (± 0.39)	1.27	0.232	0.352 (± 0.18)	2.01	0.055

Results of phylogenetic generalized least squares (PGLS). ASR is included in the models as response variable. Models 1 and 2 include snakes, birds and mammals as a single data point each; Model 3 includes all species as individual data. For sex-determination system, *b* is the estimated difference in ASR between ZW and XY species. For sex bias in dispersal, *b* is the estimated difference in ASR between the 'not male-biased' and the 'male-biased' group. *In Model 2 we used Pagel's gradual branch lengths because the model does not converge with Nee's branch lengths.

References for A/3.1

58. Myers, E. A., Rodriguez-Robles, J. A., Denardo, D. F., Staub, R. E., Stropoli, A., Ruane, S., Burbink, F. T. Multilocus phylogeographic assessment of the California Mountain Kingsnake (*Lampropeltis zonata*) suggests alternative patterns of diversification for the California Floristic Province. *Molecular Ecology*, 22, 5418–5429, (2013).
59. Rassmann, K., Tautz, D., Trillmich, F. & Gliddon, C. The microevolution of the Galápagos marine iguana *Amblyrhynchus cristatus* assessed by nuclear and mitochondrial genetic analyses. *Molecular Ecology* 6, 437 - 452 (2003)
60. Losos, J. B. *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. University of California Press, (2009)
61. Stenson A.G., Malhotra A. & Thorpe R. S. Population differentiation and nuclear gene flow in the Dominican anole (*Anolis oculatus*). *Molecular Ecology*, 11, 1679–1688, (2002)

62. Johansson H., Surget-Groba Y. & Thorpe R. S. Microsatellite data show evidence for male-biased dispersal in the Caribbean lizard *Anolis roquet*. *Molecular Ecology*, **17**, 4425–4432, (2008)
63. Calsbeek R. Sex-specific adult dispersal and its selective consequences in the brown anole, *Anolis sagrei*. *Journal of Animal Ecology* **78**, 617–624 (2009)
64. Hranitz, J. M. & Baird, T. A. Effective population size and genetic structure of a population of collared lizards, *Crotaphytus collaris*, in central Oklahoma. *Copeia*. **2000**, 786–791 (2000)
65. Zarza, E., Reynoso, V. H. & Emerson, B. C. Discordant patterns of geographic variation between mitochondrial and microsatellite markers in the Mexican black iguana (*Ctenosaura pectinata*) in a contact zone. *Journal of Biogeography*, **38**, 1394–1405 (2011)
66. Massot, M., Huey, R. B., Tsuji, J. & van Berkum, F. H. Genetic, prenatal, and postnatal correlates of dispersal in hatchling fence lizards (*Sceloporus occidentalis*). *Behavioral Ecology*, **14**, 650–655 (2003)
67. Doughty, P., Sinervo, B. & Burghardt, G. M. Sex-biased dispersal in a polygynous lizard, *Uta stansburiana*. *Animal Behaviour* **47**, 227–229 (1994)
68. Chapple D. G. & Keogh J. S. Complex mating system and dispersal patterns in a social lizard, *Egernia whitii*. *Molecular Ecology*, **14**, 1215–1227 (2005)
69. Houston, D. & Shine, R. Movements and activity patterns of Arafura filesnakes (Serpentes: Acrochordidae) in tropical Australia. *Herpetologica*, **50**, 349–357 (1994)
70. Urquhart, J., Wang, Y. & Fu, J. Historical vicariance and male-mediated gene flow in the toad-headed lizards *Phrynocephalus przewalskii*. *Molecular Ecology*, **18**, 3714–3729 (2009)
71. Qi, Y., Yang, W., Lu, B. & Fu, J. Genetic evidence for male-biased dispersal in the Qinghai toad-headed agamid *Phrynocephalus vlangalii* and its potential link to individual social interactions. *Ecol Evol.* **3**, 1219–1230 (2013)
72. Rivera, P. C., Gardenal, C. N. & Chiaraviglio, M. Sex-biased dispersal and high levels of gene flow among local populations in the argentine boa constrictor, *Boa constrictor occidentalis*. *Austral Ecology*, **31**, 948–955 (2006)
73. Chim, C. K. & Diong, C. H. A mark-recapture study of a dog-faced water snake *Cerberus schneiderii* (Colubridae: Homalopsidae) population in Sungei Buloh wetland reserve, Singapore. *The Raffles Bulletin of Zoology*, **61**(2), 811–825, (2013)
74. Pernetta, A. P., Allen, J. A., Beebee, T. J. C. & Reading, C. J. Fine-scale population genetic structure and sex-biased dispersal in the smooth snake (*Coronella austriaca*) in southern England. *Heredity*, **107**, 231–238 (2011)
75. Dubey, S., Brown, G. P., Madsen, T. & Shine, R. Male-biased dispersal in a tropical Australian snake (*Stegonotus cucullatus*, Colubridae). *Molecular Ecology*, **17**, 3506–3514 (2008)
76. Welsh, H. H., Wheeler, C. A. & Lind, A. J. Spatial ecology of the Oregon gartersnake, *Thamnophis atratus hydrophilus*, in a free-flowing stream environment. *Copeia*, **2010**(1), 75–85 (2010)
77. Hofmann, S., Fritzsche, P., Solhøy, T., Dorge, T. & Miede, G. Evidence of sex-biased dispersal in *Thermophilis baileyi* inferred from microsatellite markers. *Herpetologica*, **68**(4), 514–522 (2012)
78. Lukoschek, V., Waycott, M. & Keogh, J. S. Relative information content of polymorphic microsatellites and mitochondrial DNA for inferring dispersal and population genetic structure in the olive sea snake, *Aipysurus laevis*. *Molecular Ecology*, **17**, 3062–3077 (2008)
79. Keogh, J. S., Webb, J. K. & Shine, R. Spatial genetic analysis and long-term mark–recapture data demonstrate male-biased dispersal in a snake. *Biology Letters*, **3**, 33–35, (2007)
80. Webb, J. K. & Shine, R. A field study of spatial ecology and movements of a threatened snake species, *Hoplocephalus bungaroides*. *Biological Conservation* **82**, 203–217 (1997)
81. Croak, B. M., Crowther, M. S., Webb, J. K. & Shine, R. Movements and habitat use of an endangered snake, *Hoplocephalus bungaroides* (Elapidae): Implications for conservation. *PLoS ONE* **8**(4), e61711. doi:10.1371/journal.pone.0061711, (2013)

82. Lane, A. & Shine, R. Intraspecific variation in the direction and degree of sex-biased dispersal among sea-snake populations. *Molecular Ecology* **20**, 1870–1876 (2011)
83. Gruber, B. & Henle, K. Analysing the effect of movement on local survival: a new method with an application to a spatially structured population of the arboreal gecko *Gehyra variegata*. *Oecologia*, **154**, 679–690 (2008)
84. Olsson, M., Gullberg, A. & Tegelström, H. Malformed offspring, sibling matings, and selection against inbreeding in the sand lizard (*Lacerta agilis*). *J. Evol. Biol.*, **9**, 229–242, (1996)
85. Clobert, J. *et al.* Determinants of dispersal behavior: the common lizard as a case study. In: Vitt, L., Pianka, E., *editors*. *Lizard Ecology: Historical and Experimental Perspectives*. Princeton, *Princeton Univ. Press*; pp. 183–206 (1994)
86. Hofmann, S. Who is sitting next to me? Relatedness between next neighbours in common lizards. *Amphibia-Reptilia*, **29**, 19-24 (2008)
87. Vignoli, L., Vuerich, V. & Bologna, M A. Experimental study of dispersal behaviour in a wall lizard species (*Podarcis sicula*) (Sauria Lacertidae). *Ethology Ecology & Evolution*, **24**(3), 244-256 (2012)
88. Mabry, K. E., Shelley, E. L., Davis, K. E., Blumstein, D. T., Van Vuren, D. H. Social mating system and sex-biased dispersal in mammals and birds: a phylogenetic analysis, *PLoS ONE*, **8**(3), e57980. doi:10.1371/journal.pone.0057980 (2013)
89. Schlupp, I. & Podloucky, R. Changes in breeding site fidelity: A combined study of conservation and behaviour in the common toad *Bufo bufo*. *Biological Conservation*, **69**(3), 285-291 (1994)
90. Vos, C. C., Ter Braak, C. J. F., & Nieuwenhuizen, W. Incidence function modelling and conservation of the Tree Frog *Hyla arborea* in the Netherlands. *Ecological Bulletins*, **48**, 165-180 (2000)
91. Austin, J. D., Dávila, J. A., Loughheed, S. C. & Boag, P. T. Genetic evidence for female-biased dispersal in the bullfrog, *Rana catesbeiana* (Ranidae). *Molecular Ecology*, **12**(11), 3165–3172 (2003)
92. Holenweg Peter, A-K. Dispersal rates and distances in adult water frogs, *Rana lessonae*, *R. ridibunda*, and their hybridogenetic associate *R. esculenta*. *Herpetologica*, **57**(4), 449-460 (2001)
93. Perret, N., Pradel, R., Miaud, C., Grolet, O. & Joly, P. Transience, dispersal and survival rates in newt patchy populations. *Journal of Animal Ecology*, **72**(4), 567–575 (2003)

A/3.2: Population genetic models

This Supplementary Material presents population genetic models of the effect that genetic sex determination systems may have on the adult sex ratio. Section 1 develops results for deleterious mutations, and Section 2 for sex-antagonistic selection. All the models assume that populations are sufficiently large that the effects of drift can be neglected and the loci evolve independently. We expect the results will give good approximations for loci where the magnitude of the selection coefficient s is larger than $1/N_e$, where N_e is the effective population size of the sex chromosome under consideration. These models do not capture the effects of several stochastic processes thought to be important to sex chromosomes because of their reduced effective population sizes (Bachtrog 2006). One is Muller's ratchet, in which deleterious mutations drift to fixation on nonrecombining sex chromosomes. A second is the fixation of deleterious mutations that hitchhike with beneficial mutations that sweep to fixation at linked loci. While those processes could also contribute to biased adult sex ratios, they are complex, and an adequate analysis of their effects on the ASR is beyond what is possible here. In any event, the deterministic results developed here are a necessary foundation for more complex models.

Deleterious mutations

This section develops a simple model to estimate the impact that deleterious mutation may have on the adults sex ratio (ASR), which we define as the proportion of males in the adult population. If the sex ratio is unbiased at conception, the adult sex ratio will be

$$\text{ASR} = \frac{\bar{W}_m}{\bar{W}_f + \bar{W}_m} \quad (1)$$

where \bar{W}_m and \bar{W}_f are the relative rates of survival to adulthood of males and females. For concreteness, we develop the model assuming XY

sex determination, and at the end we generalize the results to ZW systems. For simplicity, the model assumes the loci are completely sex-linked, and that loci carried on one type of sex chromosome (e.g. the X) have no homologue on the other type (e.g. the Y) that might mask the fitness effects of deleterious mutations. Intuitively, we expect that loci in the pseudoautosomal region will have a smaller impact on the ASR.

Begin by considering X-linked loci. We assume that fitness effects are multiplicative across loci. The mean fitnesses of females and males are approximately

$$\bar{W}_f \approx \prod_i (1 - h_i s_i^f p_i) \approx \exp\left\{-\sum_i h_i s_i^f p_i\right\} \quad (2)$$

$$\bar{W}_m \approx \prod_i (1 - s_i^m p_i) \approx \exp\left\{-\sum_i s_i^m p_i\right\} \quad (3)$$

where s_i^f and s_i^m are the selection coefficients for the deleterious allele at locus i , p_i is that allele's frequency, and h_i is the dominance coefficient in females. These approximations hold when linkage disequilibrium is weak, mutation is weak relative to selection, and $s_i^f, h_i s_i^m \ll 1$.

At a mutation-selection equilibrium,

$$p_i \approx \mu_i / \left(\frac{2}{3} h_i s_i^f + \frac{1}{3} s_i^m \right) \quad (4)$$

where μ_i is the mutation rate to the deleterious allele at locus i . The form of the denominator is a result of the fact that deleterious mutations spend 2/3 of their evolutionary time in females and 1/3 in males. So we have

$$\bar{W}_f \approx \exp \left\{ -3 \sum_i \left(\frac{\mu_i h_i s_i^f}{2 h_i s_i^f + s_i^m} \right) \right\} \quad (5)$$

$$\bar{W}_m \approx \exp \left\{ -3 \sum_i \left(\frac{\mu_i s_i^m}{2 h_i s_i^f + s_i^m} \right) \right\} \quad (6)$$

Now assume that deleterious mutations are nearly or completely recessive in females ($h_i \ll 1$). Then

$$\bar{W}_f \approx 1 \quad (7)$$

$$\bar{W}_m \approx \exp \{-3 U_X\} \quad (8)$$

where U_X is the total mutation rate for X-linked genes (that is, the sum across loci of the mutation rates to deleterious alleles per gamete per generation).

These results are consistent with the well-known result that the genetic load (that is, the loss in population mean fitness) from deleterious mutation is simply equal to the mutation rate and is independent of selection coefficient⁹⁵. The factor of 3 appears because recessive mutations at X-linked loci experience selection in males in only one of every three generations.

The second class of loci we consider are genes that are carried on the Y but absent from the X. The genetic load principal applies directly to this case, and so

$$\bar{W}_f \approx 1 \quad (9)$$

$$\bar{W}_m \approx \exp \{-U_Y\} \quad (10)$$

where U_Y is the total rate of deleterious mutation for loci in this first class.

Combining the effects of X-linked and Y-linked genes (Eqs. (7) - (9)), we have

$$\bar{W}_f \approx 1 \quad (11)$$

$$\bar{W}_m \approx \exp \{-3 U_X - U_Y\}. \quad (12)$$

If the total mutation rates are small ($U_X, U_Y \ll 1$), the fitness of males relative to the fitness of females is:

$$\bar{W}_m \approx 1 - 3 U_X - U_Y. \quad (13)$$

We can now find the ASR resulting from deleterious mutation by multiplying the fitness effects of X-linked and Y-linked genes (Eqs.(9) - (12)), which gives:

$$\text{ASR} \approx \frac{\exp \{-3 U_X - U_Y\}}{1 + \exp \{-3 U_X - U_Y\}} \quad (14)$$

This result simplifies further when the total mutation rates, U_X and U_Y , are much smaller than 1:

$$\text{ASR} \approx \frac{1 - 3 U_X - U_Y}{2} \quad (15)$$

A parallel argument for ZW sex determination systems gives

$$W_f \approx \exp\{-3U_Z - U_W\}. \quad (16)$$

$$W_m \approx 1 \quad (17)$$

$$\text{ASR} \approx \frac{1}{1 + \exp\{-3U_Z - U_W\}} \quad (18)$$

$$\approx \frac{1}{2} + \frac{3}{4}U_Z + \frac{1}{4}U_W \quad (19)$$

where in Eq. (19) we again assume that the total mutation rates are much smaller than 1.

Sex chromosomes show bewildering variation across animals and plants¹⁷, which makes it difficult to make general statements about what the empirical implications of these calculations might be. To get some sense, consider the following crude estimates for U_X and U_Y in humans. The human X carries about 5% of the genes in the genome⁹⁶, and the Y carries about 0.3 % of the genes⁹⁷. The genome-wide rate of mutation to deleterious alleles is estimated to be about 1 per haploid genome per generation⁹⁸, suggesting $U_X \approx 0.05$ and $U_Y \approx 0.003$.

Using those values and Eq. (13), the equilibrium adult sex ratio is $\text{ASR} = 0.46$.

This calculation is very rough. The parameter estimates are crude and they neglect important factors such as sex-biased mutation. Further, we have made restrictive assumptions (e.g. that deleterious mutations are fully recessive). It seems unlikely, however, that refining the parameter estimates and generalizing the assumptions would lead to a sex ratio that deviates from 1/2 by more than a few percent for humans (and likely other eutherian mammals). The conclusion could be very different in other species, however, particularly if values for U_X and U_Y are much larger.

Sex-antagonistic selection

Sex-antagonistic selection occurs when alleles have opposing fitness effects on females and males. When this type of selection acts on sex-linked loci (on X or Z chromosomes), either fixation or polymorphism can result.

Fixation

Fixation is the typical outcome when dominance effects are weak and the relative fitness effects of the alleles within males are approximately equal in magnitude but opposite in sign to their relative fitnesses in females. In that case, the allele that is favored in the homogametic sex fixes because it experiences selection in the homogametic sex twice as often as in the heterogametic sex.

This outcome, however, does not automatically lead to a prediction about which sex will have higher mortality. That is because it is the evolutionary outcome depends on the relative fitnesses *within* each sex, and is independent of how much each allele affects females versus males. (We are very grateful to a reviewer for pointing this out.) This point may become clearer with a simple numerical example. Consider an X-linked locus in an XY sex determination system with two alleles, a and A . First consider the case where the viabilities are

	Allele		
	a	A	
Males:	0.9	1	
	aa Aa AA		
Females:	1	0.9	0.8

The female-beneficial allele a will fix, which leads to a female-biased sex ratio. Now consider a second case in which the viabilities are

	Allele		
	a	A	
Males:	0.9	1	
	aa Aa AA		
Females:	0.8	0.7	0.6

In this case, the female-beneficial allele again fixes, but the outcome is a male-biased sex ratio. These two examples illustrate the basic point that fixation of the allele that is favored in the homogametic sex does not necessarily lead to higher viability for that sex.

We are, however, able to make a general statement by introducing an additional assumption. Again assuming approximately equal relative allelic effects within each sex and weak or no dominance, and assume further that the optimal genotype for males has the same viability as the optimal genotype for females. (The first case above is an example.) The outcome is that female-favorable alleles fix in XY systems, causing the ASR to decrease. Conversely, male-favorable alleles will tend to fix in ZW systems, leading to an increase in the ASR.

When selection is stronger in the hemizygous sex, the allele favorable to that sex can fix. That would increase the ASR in XY systems and decrease it in ZW systems.

In sum, there are limited situations in which we can predict how fixation of alleles under sex-antagonistic selection will impact the ASR.

Stable polymorphism

Another possible outcome of sex-antagonistic selection is a stable polymorphism. In this case, there does not seem to be any simple generalization that can be made the direction in which the ASR will be biased. The range of parameters that support a stable polymorphism is fairly limited⁹⁹, however, so this may not often be an important factor in the evolution of the ASR.

To make these statements more quantitative, we consider a model for sex-antagonistic selection analyzed by Bennett⁹⁹. There are two alleles, A and a , that are X-linked. The relative viability scheme that we use here is:

$$\begin{array}{l} \text{Males:} \quad a \quad A \\ \quad \quad 1 \quad 1 + s_m \\ \\ \text{Females:} \quad aa \quad Aa \quad AA \\ \quad \quad 1 + s_{aa} \quad 1 \quad 1 - s_{AA} \end{array}$$

For concreteness here we assume all the $s \geq 0$, which means allele A is favored in males and allele a in females, and that heterozygotes have intermediate fitness in females.

Bennett found that a stable polymorphism occurs if and only if:

$$s_{aa} > \frac{s_m}{2} \quad \text{and} \quad s_{AA} < \frac{s_m}{2(1 + s_m)} \quad (20)$$

When those conditions are met, the equilibrium frequency of A in eggs is p_f (eq. 21), and in sperm it is p_m (eq. 22)

$$\hat{p}_f = \frac{2s_{aa} - s_m}{2(s_{aa} - s_{AA}(1 + s_m))} \quad (21)$$

$$\hat{p}_m = \frac{(1 + s_m)(s_m - 2s_{aa})}{s_m^2 - 2s_{aa}(1 + s_m) + 2s_{AA}(1 + s_m)} \quad (22)$$

When there is no dominance in females ($s_{aa} = s_{AA}$), one can show that the female favorable allele a will fix if:

$$\frac{s_m}{2} \leq s_{aa} = s_{AA} \quad (23)$$

That result is the basis of the earlier statement that the female-favorable allele will generally fix when fitness effects in the two sexes are of comparable magnitude and opposite in sign, and heterozygotes have intermediate viability. The conclusion is reversed for ZW systems: there Z chromosomes spend more of their evolutionary histories in males, and so male-favorable alleles will tend to fix.

When there is a stable polymorphism, we can calculate the ratio of female to male fitness:

$$R_W = \frac{(s_{AA} (1 + s_m) - s_{aa}) (1 + (2 - 4 (1 + s_{aa}) (1 - s_{AA})) (1 + s_m) + (1 + s_m)^2)}{(1 - 2 (1 + s_{aa} - s_{AA}) (1 + s_m) + (1 + s_m)^2)^2} \quad (24)$$

Here are two numerical examples. In the first, females have higher viability than males ($R_W > 1$), while in the second example the males have higher viability ($R_W < 1$):

s_m	s_{aa}	s_{AA}	p_f	p_m	R_W
0.205	0.1	0.1	0.12	0.14	1.09
0.22	0.1	0.1	0.45	0.50	0.91

These examples illustrate the point made earlier that there are no robust generalizations about which sex will have higher viability when sex-antagonism results in a polymorphism.

References for A/3.2

95. Haldane, J.M.S. The effect of variation on fitness. *American Naturalist* 71: 337-349 (1937).
96. Ross, M.T., et al. The DNA sequence of the human X chromosome. *Nature* 434: 325-337 (2005).
97. Skaletsky, H., et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 423: 825-837 (2003).
98. Keightley, P.D. Rates and fitness consequences of new mutations in humans. *Genetics* 190: 295-304 (2012).
99. Bennett, J.H. Selectively balanced polymorphism at a sex-linked locus. *Nature* 180: 1363-1364 (1957)

