Brood sex ratio variation in a colonial raptor, the Eleonora's falcon, *Falco eleonorae*

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Keywords

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Abstract

Sex allocation theory predicts that species with reversed sexual size dimorphism frequently overproduce the cheapest sex at the brood level. A sex ratio deviation from parity is frequently documented among the offspring of raptors and is largely produced by sex-biased survival or parental adjustment as a response to environmental variability or demographic conditions. The Eleonora's falcon is a long-distance migrant wintering in Madagascar and southeast Africa and breeding colonially primarily in the Mediterranean Sea and the Canary Islands. It feeds on migratory passerines and thus depends greatly on the autumn migration flow and the prevailing winds. Being sexually size dimorphic (i.e. females are larger than males) and depending greatly on the migration, the species is a good case for investigating whether brood sex ratio variation is adaptive. In the present study, we examined the proportion of males and females reared during 2009–2020 in a falcon colony in southeast Crete (Greece) in relation to specific ecological and biological attributes. Female nestlings were shown to be costlier to produce as they are heavier than males. Overall, an unbiased sex ratio was observed across the years, although a consistent trend was recorded towards females early in the breeding season and first-hatched chicks and males late in the breeding season and third-hatched chicks. Our results provide strong evidence that parental condition, habitat quality and a brood reduction effect were the significant drivers for the observed sex ratio patterns. The overproduction of the costlier sex and a high survival of the cheaper one during food-rich years should be regarded as an adaptive evolutionary strategy of the species for maximizing its fitness returns per clutch and for maintaining a balanced offspring sex ratio in the population.

Introduction

Sex allocation theory explains populations' sex ratios and combines evolutionary and behavioural approaches, together with mathematical models and empirical testing (Hardy & Boulton, 2019). The variability of sex ratio patterns can be responsive to ecological and temporal environmental conditions and quantifying them can improve our understanding of evolutionary processes as natural selection appears to determine relative differences. The sex ratio often depends on the relative costs and benefits of producing offspring of each sex and is affected by habitat quality, life history traits, competition and dispersal, following seasonal changes in the availability of resources (Sapir et al., 2008). Fisher's theory was an early model of sex allocation (Fisher, 1930) postulating that natural selection favours a balanced 1:1 sex ratio. In other words, frequency-dependent selection should lead to the equal production of females and males when the fitness of a genotype or phenotype is affected by its frequency in the population (Ayala & Campbell, 1974). Nevertheless, when the energy costs for raising male and female offspring differ, natural selection would favour an equal investment in the sexes, even if this results in the overproduction of one sex (Kalmbach et al., 2001).

In birds, sex determination was assumed for many years to be random, without being controlled by the parents (Clutton-Brock, 1991; Frank, 1990). However, over the last 50 years scientific evidence has confirmed that a large number of species have the ability to alter the amount of energy they invest in male versus female progeny and thus are able to manipulate their offspring's sex to increase their fitness (Davies et al., 2012). This behaviour could be shaped by natural selection and is achieved at a variety of developmental levels according to timing (Navara, 2018): (1) ‘primary’, i.e. the sex ratio at the egg stage, (2) ‘secondary’, i.e. the sex ratio at the nest stage and (3) ‘tertiary’, i.e. the sex ratio at the adult stage (Mayr, 1939). In nature, the majority of avian examples of skewed sex ratios have been observed at the nest stage and are connected to the existing environmental and social conditions (Navara, 2018). The sex ratio could be skewed in response to a specific parental condition (‘parental/ environmental condition hypothesis’, Trivers & Willard, 1973) where the production of the 'expensive' sex will offset its cost when resource availability and consequently foraging success are high (Davies et al., 2012; Trivers & Willard, 1973). In contrast, when foraging success is poor, parents would overproduce the ‘cheapest’ sex or at least the one that is less sensitive to harsher environments (Arroyo, 2002; Benito & González-Solís, 2007; Myers, 1978; Sheldon, 1998). Nevertheless, as fitness returns depend on the environmental conditions during offspring development, this would require parents to predict resource availability and adjust brood sex ratio accordingly by somehow dividing their parental expenditure between their offspring.

Sex ratio adjustment could evolve through the offspring's position within a brood, for example its birth date, weight, age or hatching order (Carranza, 2002 and references therein) or with seasonal shifts in parental investment operating under certain ecological factors (‘the early bird hypothesis’, Smallwood & Smallwood, 1998). Offspring loss usually occurs due to the increased mortality of the later (younger) hatchling and the increased survival of the earlier (older) one (Magrath, 1989). In adverse environmental conditions the nestlings of the larger sex, which require increased parental investment, are at greater risk of dying, which is accelerated if they come from the last egg within a clutch (Torres & Drummond, 1997). For this reason, as the proportion of food that parents provide to the nestlings usually decreases with the hatching order, the death of the last-hatched nestling is more probable if it belongs to the costlier sex (Benito & González-Solís, 2007). To prevent this unfavourable situation, the larger (‘expensive’) sex is expected to be produced at higher frequencies earlier in the hatching sequence (‘intrabrood sharing out hypothesis’, Carranza, 2004). In contrast, when there is increased intrabrood competition, it is the offspring of the smaller (‘cheaper’) sex that is at a disadvantage compared to its siblings of the larger sex and the adaptive strategy to escape sibling aggression and starvation would be to hatch earlier in the hatching sequence (‘intrabrood competitive equilibrium hypothesis’, Uller, 2006).

Apart from parental quality, the social environment such as competition between siblings might also favour the sexes unequally (Leimar, 1996; Navara, 2018). If an offspring of one sex contests its siblings for resources, the sex ratio would be biased towards the opposite sex which would be favoured by natural selection to reduce competition (‘local resource competition hypothesis’, Clark, 1978; Leimar, 1996; Julliard, 2000; Komdeur & Pen, 2002; Wild & West, 2007; Jaatinen et al. 2013). Alternatively, when the progeny of one sex is more likely to remain in or return to the natal group (e.g. colony) the sex ratio should be biased towards the philopatric sex (‘local resource enhancement hypothesis’, Davies et al., 2012). In the latter case low habitat quality would suppress the philopatric sex and the offspring sex ratio would be biased towards the more dispersive one (Komdeur et al., 1997; Sheldon, 1998).

In raptors, sex ratio deviations from parity are usually discussed in relation to their reversed body size dimorphism and the sex allocation process is facilitated by parents selecting to produce intrabrood position asymmetries which may lead to brood reduction (Slagsvold et al., 1986; Andersson, 1994; Laaksonen et al., 2004; Magrath et al., 2007; Erikstad et al., 2009; Woolaver et al. 2015). If the sexes of the offspring differ in size, competition between siblings might arise due to the higher energetic requirements of the larger sex (Fiala & Congdon, 1983) which is more exposed to disadvantageous conditions such as food scarcity (‘production cost hypothesis’, Daan et al., 1996). However, the exact mechanism seems rather complicated, especially for species that produce large broods (Davies et al., 2012; Hardy, 1997; West et al., 2002).

In the present study we focused on a monogamous raptor species, the Eleonora's falcon, which breeds colonially, raising broods of one to three nestlings, thus allowing for sufficiently large sample sizes for the tertiary sex ratio to be studied (Walter, 1979, Swatschek et al., 1993; Cramp & Simmons, 1980). So far, only one study has investigated the seasonal sex ratio variation of the species, showing an overall bias towards male fledglings with more males being born as the hatching period progresses. The observed bias was suggested to be adaptive and determined largely by experienced pairs that start clutches early and invest preferentially in female offspring (Ristow & Wink, 2004). However, as that study was carried out during a period of population decline (Ristow, 2001), density-dependent competition was relaxed. In addition, many of the best-quality nests where broods of three nestlings were raised were on inaccessible steep cliffs, while on reachable nests rat predation accounted for ca. 25% of annual egg losses (Ristow & Wink, 1985; Ristow, 1999). For these reasons some data might have been missed or underrepresented. This is especially so given that life history patterns may differ locally in line with the spatial availability of resources or weather conditions (Byholm, 2005).

Our aim was to assess the temporal variation of the sex ratio of Eleonora's falcon nestlings and investigate its relation to extrinsic factors such as environmental and habitat conditions (e.g. weather and nest site) that operate locally during the chick-rearing stage (Xirouchakis et al., 2012). The focal colony was ideal for studying sex allocation in the species as well as its association with specific ecological parameters. Accessible nests (ca. 80–95%) of various types (e.g. under bushes, potholes, rock ledges, caves, etc.) are placed on locations with different microclimate settings (e.g. sun exposure, humidity, orientation, etc.) on a rat-free islet. A previous study (Xirouchakis et al., 2012) showed that the colony has a high fledging success (94%) and a representative frequency of three-egg clutches (ca. 49%) for the species populations in the Aegean. This is important for the purpose of the study since the last egg is smaller and hatches later than the others (Ristow & Wink, 1985). As a result, the third ('marginal') chick is more susceptible to food stress than its siblings (Mock et al., 1990; Simmons, 1988). In this context we tested the major hypotheses of sex allocation theory relevant to the sex ratio in the Eleonora's falcon. We assumed that the optimal sex allocation pattern would depend on the most influential factors affecting the parents' fitness and respond to sex-specific effects on the reproductive value of their offspring (Sheldon et al., 1997). As extreme climatic fluctuations causing heat stress or prolonged windless periods, when their migrating prey are scarce, could affect the species' demography through unbalanced sex ratios, we included environmental conditions as a factor affecting the quality of the colony's nesting and foraging habitat. More specifically, we defined habitat quality by the topographic and microclimatic conditions that protect the nest from heat stress and disturbance (Ristow & Wink, 1985). Overall, we predicted that in the Eleonora's falcon the costlier and less philopatric sex, namely females (Ristow & Wink, 2004), would be overproduced: (1) during 'good' years ('production cost hypothesis'), (2) early in the breeding season ('early bird hypothesis'), (3) in first-hatched chicks ('intrabrood sharing out hypothesis') and (4) in years with a high breeding density and consequently intense intraspecific interactions, although this pattern might be impeded under strong nest site competition (Wild & West, 2007; Jaatinen et al., 2013; 'production cost hypothesis' versus 'local resource competition hypothesis'). In contrast, the cheaper and more philopatric sex, namely males, would be overproduced (1) during 'bad' years, (2) in first-hatched chicks only during increased competition between siblings ('intrabrood competitive equilibrium hypothesis'), (3) in larger clutches as the hatchlings of the opposite costlier sex would be more susceptible to brood reduction, and (4) in high-quality nests, selected by a combined effect of social circumstances, environmental conditions and parental care. Our work contributes to sex allocation theory by investigating whether parent birds can adjust their offspring sex ratio in an adaptive way and whether deviating from parity is a consistent breeding strategy in response to a variable environment. The empirical support for the selective factors that shape sex ratios would facilitate the investigation of more detailed questions about natural selection and explain ecological problems or assist in conservation practices (Hardy & Boulton, 2019; Robertson et al., 2006).

Methods

Study Species

The Eleonora's falcon is a long-distance migrant breeding on islands in the Mediterranean Sea, the Atlantic coast of Morocco and the Canary islands and overwintering in Madagascar and southeast Africa (Dimalexis et al., 2008; Kassara et al., 2017; López-López et al., 2010). The species global population is estimated at 32 400–33 300 individuals (BirdLife International, 2022) with Greece holding up to 80% of the breeding population, with an estimated 12 300 pairs largely nesting on uninhabited islets of the Aegean archipelago (Dimalexis et al., 2008, 2019). The Eleonora's falcon is the latest of all the summer visitors in Europe to breed (Dimalexis et al., 2008) switching its diet from insects to birds to exploit the autumn migration of passerines (Wink & Ristow, 2000; Xirouchakis et al., 2019). Even though individuals return to their breeding colonies in April, to occupy the most suitable territories and secure their nest sites, courtship begins in July (Ristow, 1999; Wink & Ristow, 2000). The majority of eggs are laid by mid-August. Clutch size is one to three eggs (rarely four) with three-egg clutches tending to be laid earlier in the breeding season (Ristow & Wink, 1985; Wink & Ristow, 2000). Incubation lasts approximately 28–33 days and nestlings abandon their nests when 40 days old (Dimalexis et al., 2019; Wink & Ristow, 2000). The nests are found on cliff ledges, in caves and potholes or on bare ground under bushes or boulders and how well they protect the chicks from heat stress and wind is crucial for the reproductive outcome (Vaughan, 1961; Xirouchakis et al. 2012; Xirouchakis & Panuccio, 2019). The species exhibits sexual size dimorphism (males: 327 ± 22 g; females: 399 ± 27 g, Wheeler & Greenwood, 1983; Wink & Ristow, 2000); males become sexually mature when 3–4 years old and females at 2 years old (Wink & Ristow, 2000). The falcons develop strong ties to their breeding and natal grounds, as well as with their partners; they are regarded as monogamous with no evidence for extrapair copulations (Wink & Ristow, 2000). Adults are site tenacious, returning to the same breeding colony every year, while young individuals tend to return to nest close to or at their natal colonies, thus manifesting a high degree of philopatry (Ristow et al., 1979; Ristow, 1999). In spite of that, males are more philopatric than females which are more prone to disperse and readily move to other colonies (Wink & Ristow, 2000).

Study Colony

The study was conducted on an uninhabited islet approximately 400 m offshore of southeast Crete (Fig. 1). Its area is about 1 ha with a perimeter of 620 m and a maximum altitude of 12 m above sea level. The islet is dominated by limestone cliffs and partially covered by halophilous plants, garrigues and Mediterranean shrubs (e.g. *Euphorbia dendroides*, Portolou et al., 2009; Xirouchakis & Panuccio, 2019) which provide suitable nesting locations for the falcons. The surrounding area has a Mediterranean to subtropical climate, with an extended dry period, from April to mid-November. The mean annual temperature is 20.8 °C and the total annual precipitation 300 mm (Xirouchakis & Panuccio, 2019). The population of the colony during the last two decades ranged between 80 and 120 individuals or 40–50 breeding pairs plus some floaters.

Fieldwork and Sample Collection

Fieldwork was carried out between 2009 and 2020 thus accounting for a study period of 12 years. A minimum of three field visits were made to the colony per year, each lasting 1–2 days and covering the crucial stages of the species' breeding cycle. The first visit took place during 25–28 August, when egg laying has been completed and active nests can be identified by flushing incubating falcons. On this visit, clutch size was recorded, and each nest received a unique numbered plastic board nailed on a nearby rock for identification. The second visit occurred during 5–7 September, when all nests were inspected for failed eggs while newly hatched nestlings were tallied. The last visit was made during 18–22 September when all nestlings prior to fledging were counted, marked with metal rings (obtained from the Hellenic Ringing Scheme), measured and blood sampled. Fledglings were placed into cotton bags and were weighed with a digital (5 kg) Pesola spring scale to the nearest 0.01 kg. Then two morphometric measurements were collected for each individual: (1) wing length (i.e. the distance from the carpal joint of a flattened wing chord to the tip of the longest straightened primary) measured to the nearest 1 mm with a steel stop-ruler and (2) tarsus length (i.e. the distance from the tarsometatarsal joint to the articulation of the middle toe) measured to the nearest 0.1 mm by a vernier calliper. Last, a blood sample (0.2 ml) was collected from the brachial vein of each fledgling by using an insulin syringe with an ultrafine 6 mm needle and was transferred to a capillary tube that contained 95% ethanol. If blood sampling was impossible due to the small size of the fledgling (e.g. the youngest chick in three-egg clutches), a pectoral feather was taken. All samples were stored at –20 °C for DNA extraction.

Molecular Sexing and Genotyping

For the sex determination of the chicks, genomic DNA was extracted from whole blood samples using a standard salt protocol from Miller et al. (1988), with slight modifications. Specifically, 200 μl of blood were transferred into a new tube, centrifuged at maximum speed for 15 min (4 °C) and washed 2x with 400 μl of a wash buffer (10 mM Tris PH 8/100 mM EDTA). The samples were then treated with 700 µl of a lysis buffer (0.1 M EDTA/10 mM Tris PH 8), 60 µl of 10% SDS and 10 µl proteinase K (20 mg/ml) and were incubated overnight at 55 °C. The rest of the protocol was then applied without any modifications. For feathers, the DNeasy Blood & Tissue Kit (Qiagen, Venlo, The Netherlands) was implemented to obtain DNA of high quality and quantity (Harvey et al., 2006). The PCR amplification was performed with the forward primer 1237L (5′ GAGAAACTGTGCAAAACAG 3′) and the reverse primer 1237L (5′ TCCAGAATATCTTCTGCTCC 3′), according to the protocol of Kahn et al. (1998). The PCR reaction mix (10 µl) consisted of 1 µl total DNA (30 ng), 0.3 µl of each primer (10 µM), 0.2 µl KAPA dNTP mix (10 mM), 1 µl KAPA buffer (10x), 0.6 µl MgCl2 (25 mM) and 0.04 µl Taq polymerase (Kapa Biosystems, Wilmington, MA, U.S.A.). Each PCR reaction started with an initial denaturation step at 94 °C for 2 min followed by 30 cycles of 30 s at 94 °C, 60 s at 56 °C and 2 min at 72 °C and a final extension step for 2 min at 72 °C. PCR products were separated with electrophoresis using a 3% agarose gel stained with MIDORI Green Xtra (Nippon Genetics, Düren, Germany). Double bands represented a female individual and a single band a male (Griffiths et al., 1998). To verify the results obtained with gel electrophoresis, 50 samples were genotyped for the CHD1 gene using a fluorescently labelled forward primer (6-FAM 1237L). The labelled PCR products were then run on an ABI PRISM3730 sequencer (Applied Biosystems, Waltham, MA, U.S.A.) along with GeneScan 500 LIZ dye size standard and raw allele sizes were scored with the STRand software (v.2.4.59, http://www.vgl.ucdavis.edu/STRand). The samples that had two bands on a 3% agarose gel and were characterized as females had two peaks, at 296 bp and 318 bp, in a chromatograph. The single-band samples that were characterized as males had a single peak at 296 bp (Fig. 2).

Data Processing

The age of fledglings was calculated by a growth curve formula which converts the wing chord into hatching date with an accuracy of ±1 day (Ristow & Wink, 2004; Wink et al., 1991). Once the age of individuals was assessed, their hatching date was determined by back counting from the date their wing was measured. The age of the third young, which experiences a lower growth rate than its older siblings, was adjusted so as not to exceed its maximum hatching interval from the second one, i.e. 5 days (Wink et al., 1985). The range and average age of all fledglings were used to split the hatching period into three timeframes according to the median hatching date (24 August) namely (1) before 22 August (early = 1), (2) during 22–26 August (median = 2) and (3) after 26 August (late = 3). Clutch size and brood size corresponded to the number of eggs laid and the number of hatchlings in a nest, respectively. A successful nest was defined as any nest where a clutch was laid and at least one chick was fledged. We ranked siblings in a brood according to their hatching sequence. The third-hatched nestling was easily identified by its smaller size and body mass whereas the order of the first and second to hatch was based on their plumage, that is the growth of head and body feathers, and their weight (Ristow & Wink, 2004; Ristow et al., 2004; Telailia et al., 2013) and was corrected by their calculated age (<1% of the cases were misclassified). Since fledglings were ringed at different ages their body condition was calculated as the standardized residuals (z scores) from a linear regression of weight on a structural size (i.e. weight = 2.39 × wing length + 52.6, $F_{1,533} = 1710$, $R^2 = 0.76$, $P < 0.001$; Velando et al. 2001; Everitt & Skrondal, 2010, Jaatinen et al., 2013; Tschumi et al., 2019). To assess any interannual effects of sex on size dimorphism, the body mass (g) of fledglings was explored in relation to clutch and brood size and their age was standardized between the study years in accordance with their hatching date. Furthermore, as the data were collected during three different developmental stages (i.e. egg, hatchling, fledgling), the analysis was applied on (1) a 'full' data set, namely all the successful nests that experienced partial egg or hatchling losses, (2) a 'clutch' subset, where only nests with zero egg and hatchling losses were taken into account and (3) a 'brood' subset, where nests with all hatchlings surviving to fledging were considered. Our results refer to the tertiary sex ratio (Mayr, 1939) which was calculated as the proportion of males among fledglings.

For habitat quality three different topographic variables were used for each nest: (1) the location on the islet (sector), (2) the nest category (type) and (3) the direction of the nest entrance (orientation). Nests were divided into three quality categories (i.e. high, medium and low) based on a nest site quality score (N_Q) that was calculated by the equation $N_Q = (0.25 \times \text{sector}) + (0.5 \times \text{type}) + (0.25 \times \text{orientation})$, thus giving more weight to nest structure (type) as protection against heat stress. Sector was set as a factor of four levels (sector index 1–4) corresponding to different parts of the island with different temperature and humidity conditions (Xirouchakis, 2022): level 1 was the most, and 4 the least, beneficial for reproduction. Similarly, nest type was set as a factor of seven levels describing nest topology: open ground, under a bush, rock ledge, pothole, under a boulder, cave/pigeonholes of highly weathered cliff and burrow (type index values of 1, 1.5, 2, 2.5, 3, 3.5 and 4), reflecting increasing protection against heat stress. Orientation was indexed according to the air-cooling conditions of the prevailing winds (orientation index): N/NE/NW = 4, E = 3, S/SW/SE = 2, W = 1.

Hatching success was calculated as the proportion of eggs hatched per clutch, fledging success as the proportion of young fledged per young hatched, breeding success as the proportion of young fledged per clutch, productivity as the number of fledglings per breeding attempt and fledging rate as the number of fledglings per successful pair (Xirouchakis et al., 2012). A pair was considered successful if at least one young was present in the nest from mid-September onwards (Ristow, 1999). We accounted for environmental conditions and regarded as 'bad' years those breeding seasons with a fledging rate below 1.8 fledglings, which is the average value for south Aegean colonies around Crete (Xirouchakis et al., 2012). We also examined sex ratio in relation to population density by using the nest's nearest-neighbour distance (NND) and assuming that a crowded colony would experience adverse social interactions (e.g. food piracy; Ristow, 1999). Parental quality was indirectly evaluated by clutch size assuming that old, experienced breeders would be more successful in raising young (Xirouchakis et al., 2012). Good-quality parents were regarded as those laying three-egg clutches given that older males produce more nestlings and usually mate with experienced females (Badami, 1998; Wink & Ristow, 2000).

Statistical Analysis

Initially all continuous data (i.e. proportion of males, age, body mass) were presented as means ± SD. Body mass and body condition were explored for normality and homogeneity of variances by using the Shapiro-Wilk's statistic and the Levene's test, respectively. When normality was violated, nonparametric analysis of variance or t tests were applied (i.e. Kruskal–Wallis test followed by post hoc pairwise comparisons or Wilcoxon test with a Bonferroni correction). To compare the observed and expected proportions of males at nests each year and for all years combined we used exact binomial tests assuming a sex ratio of parity (Mangiafico, 2015). To evaluate the sex allocation pattern in relation to hatching period and sequence, clutch and brood size as well as habitat quality, we used *G* tests (Zar, 2009). All statistical tests were performed at a 0.05 level of significance using the software package R 4.0.3 (R Core Team, 2020) and relevant libraries i.e. 'stats', 'pgirmess', 'RVAideMemoire' (Giraudoux, 2009; Hervé, 2021). Nest orientation was measured as compass direction and was transformed to angles. Relevant differences between nests producing males or females were analysed by the Rayleigh uniformity test and the departure of their directional distribution from a perfect circle was tested by the *K* concentration index (Fisher et al., 1993; Mardia & Jupp, 2000). For circular data we used the Oriana 2.02 software (Kovach Computing Services, 2005).

The influence of intrabrood structure (i.e. age, hatching order), habitat and parental quality on sex allocation was assessed by two generalized linear mixed models (GLMMs) constructed with a logit-link function and a binomial error distribution (Pinheiro & Bates, 2000; Faraway, 2006). In the first model the sex of individual fledglings was set as a binary response variable (0 = male, 1 = female) while hatching period, hatching sequence, brood size and nest quality score were fitted as explanatory variables (i.e. fixed-effects factors). Nest identity nested in time (i.e. year) was included as a random factor to account for many nestlings within broods over multiple years. In the second model the annual proportion of males was set as the response variable while population density (i.e. mean NND), environmental conditions (i.e. mean fledging rate), habitat quality (i.e. mean nest quality score) and parental quality (i.e. percentage of three-egg clutches) were set as explanatory variables. Time (i.e. year) was added as a random effect. Nests were analysed by their overall quality scores as well as by their individual indexes, i.e. sector, type and orientation. In the response variable, the number of males and number of females were bound in a two-vector response variable so as not to lose information of the sample size from which the proportion of sexes was estimated (Crawley, 2012). Body condition data were evaluated after a square-root transformation (for negatively skewed data) so negative and positive values were treated symmetrically. The outcome was a series of calculated values that were regressed on sex, age, sequence of hatching and brood size by a linear mixed model with a gamma error distribution and a log-link function (suitable for continuous, strictly positive response variables). Nest identity nested in year was set as a random factor (Crawley, 2012).

All explanatory variables were checked for multicollinearity by running a variance inflation factor (VIF) test (Aguilera et al., 2006) and were all accepted because VIFs were less than 1. Models were constructed with the R library 'glmmTMB' (Bates et al., 2015; Brooks et al., 2017) and selected by a stepwise procedure where nonsignificant factors and their interactions were removed by using the Akaike information criterion (AIC; Akaike, 1980). The levels of significance (P values) for the variables included in each step of model selection were also examined while the final models that possessed the lowest AIC were tested for overdispersion.

Ethical Note

Fieldwork was performed taking the welfare of the birds into account. On the first two visits, nest checks took up to 5 min, while on the third visit measurements, ringing and blood sampling took ca. 15 min per nest. Unnecessary stress was reduced during these latter manipulations by keeping birds in cotton bags and under shade with the least possible noise. The ringing and blood sampling of Eleonora's falcons were authorized by the Hellenic Ministry of Environment and Energy (permit no. 176962/2386, 180468/657). The Natural History Museum of Crete (scientific institution code GR002), possesses a CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) sampling permit (ref. no. 096860/2199/23-8-2005). All ringed falcons were provided with water before being placed in the nest cavity. A certain level of stress was caused to fledglings by handling and their parents were alert, flying over the nest, but they all relaxed as soon as fieldworkers walked away from the nesting territory. No nest failure or premature fledging caused by the fieldworkers was recorded during the study.

Results

Sampling and Sexing

Over the 12 consecutive years of this study, 98 Eleonora's falcon nests were monitored in which 259 clutches were laid. Of these, 146 (56.4%) contained three eggs, 108 (41.7%) contained two eggs and only five (1.9%) contained one egg. Of all clutches, 38.2% (N = 99) experienced an egg loss and a further 16.2% (N = 42) suffered an extra brood reduction. Overall, 535 fledglings were captured in nests, which represented 86.3% of the colony's progeny for the study period.

Clutch Size and Brood Reduction

No annual variation in clutch size frequencies was detected throughout the study period or between nests of different type, orientation and quality. Similarly, no significant proportional differences were detected between broods suffering partial losses and those remaining intact until fledging. However, clutch and brood size frequency differed between the colony sectors with three-egg clutches and broods of three nestlings being more frequently found in the cooler, than the hotter, sectors of the islet (G test: clutches: $G_6 = 13.8$, $P < 0.05$; broods: $G_6 = 15.4$, $P < 0.05$). Similarly, more three-egg clutches were laid early in the breeding season (G test: $G_2 = 129.5$, $P < 0.001$) while mean clutch size declined with increasing laying date (Kruskal–Wallis test: $H_2 = 16.2$, $P < 0.001$). Irrespective of clutch size, 18.9% of all the eggs laid and 5% of all the nestlings hatched were lost. Nest failures mostly accounted for the last egg or nestling although hatchling mortality might have been underestimated as many third-hatched nestlings often vanished soon after hatching (S. Xirouchakis, personal observation). In any case nests under heat stress on average suffered greater brood losses than those in cool, shaded sites (Kruskal–Wallis test: $H_3 = 16.7$, $P < 0.001$; pairwise Wilcoxon post hoc tests: $P < 0.05$).

Sex Ratio Temporal Pattern

Regarding sexing, 268 fledglings were males and 267 were females, resulting in an overall sex ratio of 1:1. The mean annual proportion of males across broods was 0.52 ± 0.08 (range 0.37–0.62; Table 1). In eight of the 12 study years (66.7%), the sex ratio was slightly male biased, although no significant deviance from parity was detected between or within years (binomial test: $P > 0.05$; Table 1). Almost half of the nests (48.6%) with two- and three-egg clutches produced fledglings of mixed sexes with a 1:1 sex ratio. Likewise, no sex ratio bias was detected in nests where all clutches and broods survived to fledging.

Within-brood Sex Ratio

A sex ratio bias was detected when the hatching date of the fledglings was checked. On average young falcons were ringed at the age of 25 ± 3.8 days for males (range 10–34) and 27 ± 4.1 days for females (range 12–37) with the youngest individuals being males (Kruskal–Wallis test: $H_{25} = 54.3$, $P < 0.01$). The brood sex ratio departed significantly from parity and was biased only for the early and the late hatching period (binomial test: early: $P < 0.001$; late: $P < 0.001$). In fact, the proportion of males was significantly lower among nestlings that hatched early and significantly higher among nestlings that hatched late in the breeding season (*G* test: $G_2 = 22.6$, $P < 0.001$; Fig. 3a). The sex ratio was obviously biased across the hatching sequence (*G* test: $G_2 = 17.9$, $P < 0.001$; Fig. 3b) with a strong skewed pattern towards females and males among first- and second-hatched nestlings, respectively (binomial tests: $P < 0.01$). In contrast third-hatched nestlings had a balanced (1:1) sex ratio (binomial tests: $P > 0.05$). A similar sex ratio pattern according to the hatching sequence was also noted for the subsets of nests where all clutches (*G* test: $G_2 = 24$, $P < 0.001$) and broods (*G* test: $G_2 = 16.1$, $P < 0.001$) survived to fledging (binomial tests: $P > 0.05$). In addition, more first-hatched female nestlings were produced in early clutches (*G* test: $G_2 = 16.7$, $P < 0.001$; binomial test: $P < 0.001$). No significant relationship was found between the percentage of early-hatched chicks, of either sex, and population density (Pearson correlation: males: $r = -0.56$, $P > 0.05$; females: $r = 0.56$, $P > 0.05$). In all GLMM models, age was a better predictor of sex than the hatching date. This is a rather common effect when binning a continuous variable (age) into categories (hatching periods) and can be problematic when it comes to regression because it might result in loss of information (Altman & Royston, 2006). However, we used the hatching date instead because it derived from age and data dichotomization enhanced their plotting (Altman & Royston, 2006). Overall, the hatching date and sequence as well as nest orientation proved to be the best predictors for sex (Table 2). In fact, the probability of producing males increased with increasing orientation and hatching order and decreased with increasing age and brood size (Table 2). Last, in contrast to our prediction, larger broods were more likely to produce females, although this pattern was recorded only in nests with no brood reduction.

Habitat Quality and Sex Ratio

Nest site quality and nest type had no effect on the overall sex ratio (Kruskal–Wallis test: H_{11} = 19.7, $P = 0.05$; G test: $G_5 = 3.9$, $P > 0.05$). In contrast, a significantly higher proportion of females was detected in nests located on the NE–SW axis compared to males found in nests uniformly distributed over the colony islet (G test: $G_7 = 16.3$, $P < 0.05$; males: $K = 0.076$; females: $K = 0.22$; Rayleigh's uniformity test for females: $Z = 6.34$, $P < 0.01$). Similarly, the probability of producing females was higher in the cooler sectors of the colony where conversely significantly more males were found in nests exposed to high temperatures in the hottest sectors (G test: $G_2 = 10.5$, $P < 0.05$). The proportion of males was not affected by parental quality or nest features but decreased with increasing fledging rate which reflects the effect of environmental conditions (GLMM estimate = −0.43, Z = −2.29, $P < 0.05$). Female fledglings outnumbered males during ‘good’ years (t test: $t_{8.6} = -3.17$, $P < 0.05$) and increased with increasing breeding density, although this trend was not statistically significant (Pearson correlation: $r = 0.51$, $P > 0.05$; Fig. 4).

Offspring Sex and Body Condition

On the day of ringing all fledglings had an average body mass of 456.9 ± 74.2 g (range 140–610, $N = 535$), with females being significantly heavier than males (females: 482 ± 68.9 g; males: 431 ± 70.8 g; Wilcoxon test: $W = 20\,112$, $P < 0.001$). This difference remained significant when body condition was examined implying that female fledglings were costlier to produce than males by 11%. Based on this sex-related body mass difference an adaptive sex ratio should be 56% males which does not represent a significant deviation from the observed one (binomial test: $P > 0.05$).

The body condition of fledglings was not affected by clutch or brood size, but it was significantly different between early and late hatchlings and with hatching order within a clutch (Kruskal–Wallis tests: hatching period: $H_2 = 9.3$, $P < 0.01$; hatching order: $H_2 = 20.4$, $P < 0.001$ and post hoc pairwise comparisons). Within the sexes, third-hatched males were in worse condition (Kruskal–Wallis test: $H_2 = 9.2$, $P < 0.01$), whereas the body condition of female fledglings was similar irrespective of their age or hatching order. Overall, female fledglings were in significantly better condition than their male counterparts for all hatching periods and sequences (Fig. 5). Body condition was found to be better explained by a simple random intercept model with time as the fixed factor; in this model body condition was significantly different between years (GLMM estimate = 0.004, Z = 3.30, $P < 0.001$), sexes (GLMM estimate = −0.053, Z = 6.41, $P < 0.001$) and hatching order (GLMM estimate = 0.016, Z = 2.87, $P < 0.01$). However, the examination of clutches surviving to fledging showed an effect of age on the body condition of young falcons which depended on their hatching order. Specifically, body condition improved with age apart from the third marginal young for which a negative relationship between body condition and age was noted (Fig. 6).

Discussion

Consistent with the only other relevant study on the species (Ristow & Wink, 2004) the sex ratio of Eleonora's falcon at fledging in the present study did not differ from parity, although a significance bias in relation to the onset of egg laying was confirmed. Female nestlings were more abundant early in the hatching period, while males dominated in the late hatching period. However, the present study went beyond seasonal variation in brood sex ratio, showing that the hatching sequence, the breeding habitat and food availability could also predict the sex of the Eleonora's falcon at fledging. First-hatched nestlings were mostly females whereas second- and third-hatched nestlings were likely to be males but only in nests with no partial losses, a result that suggests a brood sex ratio adjustment. Similarly, clutch and brood size frequencies were found to be constant across the years with a strong skewed sex ratio in relation to age and hatching order already present at egg laying. In contrast to the general trend for sexually dimorphic species, a brood reduction effect was noted in larger broods, with the underproduction of males implying that a sex ratio bias towards the ‘cheaper’ sex could be important in large broods but only over a certain size (Øigarden & Lifjeld, 2013). Moreover, and despite the small size of the colony's islet, the study also found significant evidence for a nest site quality effect on brood sex ratio. In particular, shaded nests with a cool microclimate, exposed to the prevailing northeasterly winds of the Greek summer (‘etesian’) contained more females. In contrast, low-quality nests in the hot and driest parts of the islet overproduced males. Ambient humidity should play a role in egg water loss and interact significantly with temperature (Veldsman et al., 2020). Most probably, the moisture in cool colony sectors impeded egg water loss by diffusion through the eggshell to the outside more humid air (Mortola, 2009). This pattern was also found in nest orientation which proved to be a good predictor of the progeny's sex, with more females being born in nests facing northeast and southwest which were well protected against heat stress.

As males are lighter than females even after fledging, the observed overproduction of males during food-poor years supports the parental condition hypothesis whereby the cheaper sex is less susceptible to environmental change, habitat quality or food-provisioning rates (Benito & González-Solís, 2007; Ferrer & Bisson, 2003; Morandini et al., 2020). Another significant selective factor for the observed sex ratio variation would be local resource competition (Gotwaty, 1993). If brood sex ratio was biased towards the sex that affects the future reproduction of parents (e.g. nest site rivalry), natural selection would favour the overproduction of the dispersive sex to relax competition in the colony. In the present study, if this hypothesis held true a sex ratio bias towards the philopatric males would be observed during ‘good’ years or in high-quality nests. In contrast, a sex ratio bias towards the dispersive females was noted during ‘good’ years and in the best nesting habitat of the colony. Nevertheless, during years of high breeding density increased levels of competition among breeders for suitable nest sites would emerge and the overproduction of females would be expected. This trend (although not significant) was indeed detected during ‘good’ years suggesting that intraspecific competition for resources did occur in the colony but it was not strong enough to cancel the production of the costlier sex by high-quality parents or during favourable foraging conditions.

Extreme bias towards the energetically smaller sex under adverse foraging conditions or towards the larger sex early in the reproductive cycle on food-rich territories has been demonstrated for various bird species including parrots, gulls and raptors (e.g. *Strigops habroptilus*, *Larus fuscus*, *Circus pygargus*, *Strix aluco*, *Falco sparverius*, *Falco tinnunculus*, *Falco peregrinus*; Weatherhead, 1983; Dijkstra et al., 1990; Olsen & Cockburn, 1991; Wiebe & Bortolotti, 1992; Sheldon, 1998; Nager et al., 2000; Korpimäki et al., 2000; Clout et al., 2002; Arroyo, 2002; Navara, 2018). On the other hand, a seasonal sex ratio bias among many raptor species regardless of food abundance has been attributed to differences between the sexes at the age of first breeding (Daan et al., 1996; Komdeur et al., 1997; Komdeur & Pen, 2002; Navara, 2018; Newton, 1979; Olsen & Cockburn, 1991; Sheldon, 1998; Smallwood & Smallwood, 1998). In small falcons (e.g. *F. tinnunculus*, *Falco naumanni*) males, but not females, that hatch early in the breeding season have a higher probability of breeding as yearlings (Komdeur & Pen, 2002). The opposite pattern has been recorded in larger species (e.g. *Accipiter nisus*, *Accipiter gentilis*, *Circus aeruginosus*) suggesting that early hatching most likely leads to successful reproduction for the sex that matures earlier (Daan et al., 1996; Dijkstra et al., 1990; Sheldon, 1998). This ‘early bird hypothesis’, which is rather a modification of the Trivers and Willard (1973) theory on parental condition, the timing of parental investment rather than its amount is critical for sex allocation (Smallwood & Smallwood, 1998). Early breeders are regarded as better in terms of physical condition and invest in the costlier sex while the sex that establishes the breeding territory is at an advantage over its competitors if hatched early. In terms of demography, age at first breeding would provide a buffer effect on population fluctuations and, in consequence, on population persistence (Rueda-Cediel et al., 2018). In the case of the Eleonora's falcon, life history traits and their consequences for fitness seem the most plausible explanation for the observed seasonal shifts in sex allocation. Female falcons are primarily responsible for nest defence, where establishing a nesting territory early in the breeding season is critical for the outcome of the breeding attempt (Wink & Ristow, 2000). Females also tend to breed at a younger age than males and their body size decreases with increasing age whereas the opposite pattern occurs for males (Ristow et al., 1989; Wink & Ristow, 2000). As the probability of breeding first as a yearling decreases with increasing birth date, hatching early would be more advantageous to females than males (Daan et al., 1996; Komdeur et al., 1997; Newton, 1998) and female falcons would possess a competitive advantage in selecting the best nest sites first (Badami, 1998; Ferrer & Bisson, 2003; Morandini et al., 2020; Wink et al., 1985, 1991, Wink et al., 1991). Furthermore, our findings support the intrabrood sharing out rather than the competitive equilibrium hypothesis. As the proportion of food provisioning usually decreases during periods of reduced food supply, females would be more vulnerable to sibling aggression and more sensitive to starvation if hatched late in the clutch (Benito & González-Solís, 2007;

Torres & Drummond, 1997; Wink et al., 1991). This is consistent with other sexually dimorphic raptors (e.g. *Haliaeetus leucocephalus*, *F. peregrinus*) where the first egg in the clutch produces predominantly females as the parents try to minimize sibling aggression against the costlier sex if this hatches last within the brood (Bortolotti, 1986; Olsen & Cockburn, 1991; Velando et al., 2002).

In general, the study confirms that sex allocation to the less expensive sex appears to constitute an evolutionarily stable strategy in species with sexual size dimorphism where the costs of rearing males and females are not equal (Benito & González-Solís, 2007; Dijkstra et al. 1998; Weatherhead, 1983). The exact mechanism of sex ratio adjustment is still debated, that is, whether a skewed sex ratio reflects functional adaptations in response to environmental and social parameters or is just a consequence of maternal reproductive constraints (Alonso-Alvarez, 2006; Navara, 2018). The manipulation of the sex ratio might be the result of energetic constraints suffered by mothers in poor physical condition that attempt to produce more offspring of the larger sex. Such restrictions might result in a limited amount of lipid accumulation in the egg yolk which could lead to the production of other substances (i.e. hormones) that can alter the sex of the egg (Alonso-Alvarez, 2014; Kappes et al., 2015). In this case, sex determination would be linked to a reproductive constraint rather than to a fitness-maximizing adaptation of the parents. Besides, females as the heterogametic sex could control the sex of individual eggs through preovulation mechanisms (Kalmbach et al., 2001; Komdeur & Pen 2002). Epigenetic regulation might also result in binding of specific proteins to the chromosomes and modify their potential during meiotic division (Navara, 2018) or predestine oocytes to maintain a particular sex chromosome (Badyaev et al., 2006). However, if these assumptions apply, preovulation adjustment would affect the entire clutch indiscriminately (Emlen, 1997). In contrast, postovulation sex selection mechanisms would favour allocation of the costlier sex with the highest fitness returns to the egg with the lowest probability of mortality, thus securing the energy invested for its production (Emlen, 1997; Komdeur & Pen, 2002). This strategy could be performed through maternal hormones that potentially influence the sex at ovulation (Komdeur & Pen, 2002; Navara, 2018). In the case of the Eleonora's falcon, extrinsic ecological factors during egg laying, such as temperature or food stress, could affect the sex of an egg by altering a variety of hormones or immunological and physiological factors deposited in an egg during yolk formation (Badyaev et al., 2006; Navara, 2018; Saino et al., 2008; Young & Badyaev, 2004). The latter scenario seems more reasonable considering that insect food availability prior to egg laying is positively correlated with clutch size (Xirouchakis et al., 2012), and the first egg that contains the costlier sex is the heaviest within a clutch (Wink et al., 1991). After egg laying, sex manipulation might occur via sex-specific egg loss or brood reduction such as a high mortality of the last egg or by the selective elimination of the marginal nestling either by starvation or by sibling aggression (Clotfelter, 1996; Wiebe & Bortolotti, 2000; Clifford & Anderson, 2001; Ferrer, 2001; Woolaver et al. 2015; Steen et al., 2012). Not surprisingly, most of the partial failures in the Eleonora's falcon nests occur during the hatching period due to infertility of the third-hatched egg or the loss of the marginal hatchling. In this framework, the purpose of the last small egg could be justified under the ‘resource-tracking’ motives of overproduction, that is, food abundance would be negatively correlated with sibling aggression, allowing the successful raising of marginal nestlings only in food-rich years (Alexander, 1974; Mock & Forbes, 1995). However, this prediction has been mainly addressed for species with obligate siblicide, a behaviour that has not been documented as adaptive in the Eleonora's falcon, although intraspecific predation and nonparental infanticide have been recorded in this species' nests during periods of food scarcity (Gangoso et al., 2015; Hadjikyriakou & Kirschel, 2016). In that case sex ratio is manipulated through egg size and mass which reflect maternal investment (Bowers et al., 2014; Love & Williams, 2011; Wu et al., 2010). However, life history theory predicts that a female investing in egg production would consequently have less to invest in parental care (Sibly et al., 2012). Therefore, a sex allocation strategy after hatching should be carried out by males. The early posthatching period is favoured as the critical period for brood reduction because nestlings reach half the adult body mass when 4 days old (Telailia et al., 2013), while the increase in body mass is not related to their hatching order (Vardanis, 2008). At this stage female falcons attend the brood and defend the nest whereas the males do all the hunting (Walter, 1979). From this perspective, parental resource allocation could be suboptimal during unfavourable weather conditions and a brood reduction effect would take place in relation to parental quality and investment. This seems an economical solution, as males could regulate mortality early after hatching and save energy that would be lost if invested in doomed nestlings in the forthcoming weeks. So overall, the observed sex ratio bias is produced by a parental sex allocation strategy where the proximate factors that determine the sex ratio at fledging are intrinsic and environmental operating at different stages of the breeding cycle. A prelaying mechanism functions through maternal condition and affects clutch size and the differential female investment in eggs while an environmental mechanism is operating through paternal quality affecting nestling survival.

In a broader context, brood sex ratio variation in living organisms could be attributed to sex-specific costs of reproduction and sensitivity to ecological constraints or resource scarcity (Bürli et al., 2022; Field et al., 2013). However, it will always be difficult to detect general taxonomic patterns and explain the facultative adjustment of offspring sex allocation by individuals across species and populations in response to environmental conditions (West et al., 2002). Relatively simple models are often able to predict patterns in empirical data for a limited number of taxonomic groups (Williams, 1979; Charnov, 1982; Chapuisat & Keller, 1999; West et al. 2002). Specifically, several studies of taxa with chromosomal sex determination and sexual dimorphism (e.g. mammals, birds, reptiles, fishes) have been shown to respond to environmental heterogeneity with shifts in offspring sex ratios consistent with adaptation (Creel et al., 1998; Kruuk et al., 1999; Nager et al., 1999; Olsson & Shine, 2001; Sakisaka et al., 2000; Sheldon et al., 1999). The tertiary sex ratio pattern of the Eleonora's falcon could be explained by a differential mortality between the sexes and the species' effort to safeguard the sex that costs more to raise and has higher mortality due (1) to the expression of recessive deleterious alleles affecting mostly the heterogametic sex, (2) to ecological stressors related to reproduction or dispersal, or (3) to intraspecific competition for resources (e.g. nests) linked with dominance (Daan et al., 1996; Donald, 2007; Kruuk et al., 1999). In any case, understanding sex ratio patterns is important not only for revealing life history traits of a species, which depend upon complex evolutionary trade-offs and interactions with the external environment, but also for applying appropriate conservation measures during the current period of environmental change (Wedekind, 2012). Since the Eleonora's falcon is expected to be highly vulnerable in a moderate climate change scenario (Dimalexis et al., 2019) and there is currently little research in relation to the sex ratio patterns of its breeding colonies, more studies on this topic should be designed. An attempt to assess offspring sex ratio in a variety of highly accessible colonies across Greece, preferably on a multiyear basis, should be highly advantageous. This could be especially useful in conservation terms in relation to global warming. Increased levels of heat or severe fluctuations in the existing wind systems in the Aegean might not only cause nest failures in the species' colonies and lower its reproductive output but also produce an unbalanced sex ratio in the offspring affecting almost its entire population.

Author Contributions

S.X. conceived the idea of the study. Data were collected in the field by S.X., K.B. and G.A. D.T. and P.B. contributed to the study design, material preparation and genetic analysis in the laboratory. Statistical analysis was performed by S.X., P.B. and D.T. A first draft of the manuscript was prepared by P.B. The submitted version of the manuscript was written by S.X. All authors read, commented on and approved the final manuscript.

Data Availability

The samples are owned by the Natural History Museum of Crete/ University of Crete. Data can be provided upon request.

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Tables

Table 1. Observed annual sex ratio (males:females) in fledglings of Eleonora's falcon in Crete

Year	**No. of clutches**	**Male**	**Female**	**Sex ratio**	**% Males**	***P*[a]**
2009	12	7	12	1:1.71	36.8	0.36
2010	16	14	10	1:0.71	58.3	0.54
2011	15	15	13	1:0.87	53.6	0.85
2012	22	13	8	1:0.62	61.9	0.38
2013	12	20	15	1:0.75	57.1	0.50
2014	14	24	20	1:0.83	54.5	0.65
2015	15	30	40	1:1.33	42.9	0.28
2016	21	27	33	1:1.22	45.0	0.52
2017	21	35	49	1:1.40	41.7	0.15
2018	22	17	11	1:0.65	60.7	0.34
2019	24	34	31	1:0.91	52.3	0.80
2020	39	32	25	1:0.78	56.1	0.44
Overall	410	268	267	1:1	50.1	0.44

[a] Binomial test.

Table 2. Factors affecting the sex of Eleonora's falcon broods in relation to the nest outcome from egg laying to fledging (i.e. nests with partial egg or hatchling losses, nests with intact clutches with zero egg and nestling losses, nests with intact broods with zero nestling losses)

	Estimate	**95% CI**	***P***
Nests with partial losses			
Intercept	1.12	0.04 to 2.193e+00	0.04
Late hatching	−0.88	−1.32 to −0.04	<0.001
Median hatching	−0.73	−0.03 to −1.16	0.001
Hatching order	−0.46	−0.73 to −0.02	<0.001
Nest orientation	−0.002	−0.003 to −9.56e-05	0.04
Nests with intact clutches			
Intercept	2.14	1.38 to 2.90	<0.001
Late hatching	−1.23	−1.77 to −0.68	<0.001
Median hatching	−0.78	−1.35 to 0.25	0.004
Hatching order	−0.55	−0.85 to −0.24	0.001
Nest orientation	−0.003	−0.006 to −0.001	0.003
Nests with intact broods			
Intercept	0.44	−0.50 to 1.38	0.36
Late hatching	−0.63	−1.11 to −0.14	0.01
Median hatching	−0.57	−1.06 to 0.08	0.02
Hatching order	−0.60	−0.90 to −0.30	<0.001
Brood size	0.49	0.13 to 0.84	0.007
Nest orientation	−0.004	−0.0004 to −0.00008	0.04

CI: confidence interval

Figures

Figure 1. Location and map of the study area.

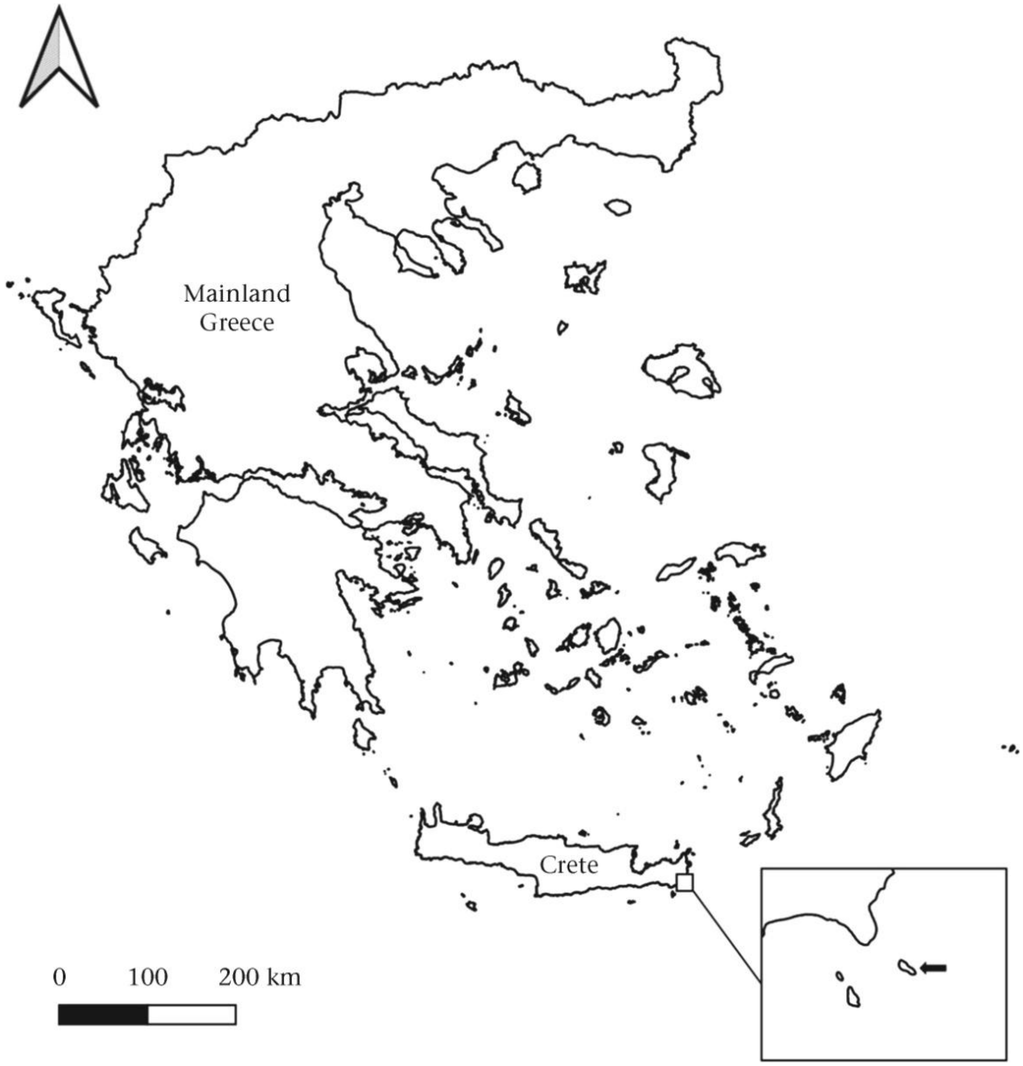

Figure 2. Chromatographs of an ABI Capillary Electrophoresis for the CHD1 gene: (a) a male individual has two CHD1 fragments of 296 bp each, resulting in a single peak, and (b) a female individual has two CHD1 fragments: a 296 bp fragment and a 318 bp one, resulting in two peaks in a chromatograph.

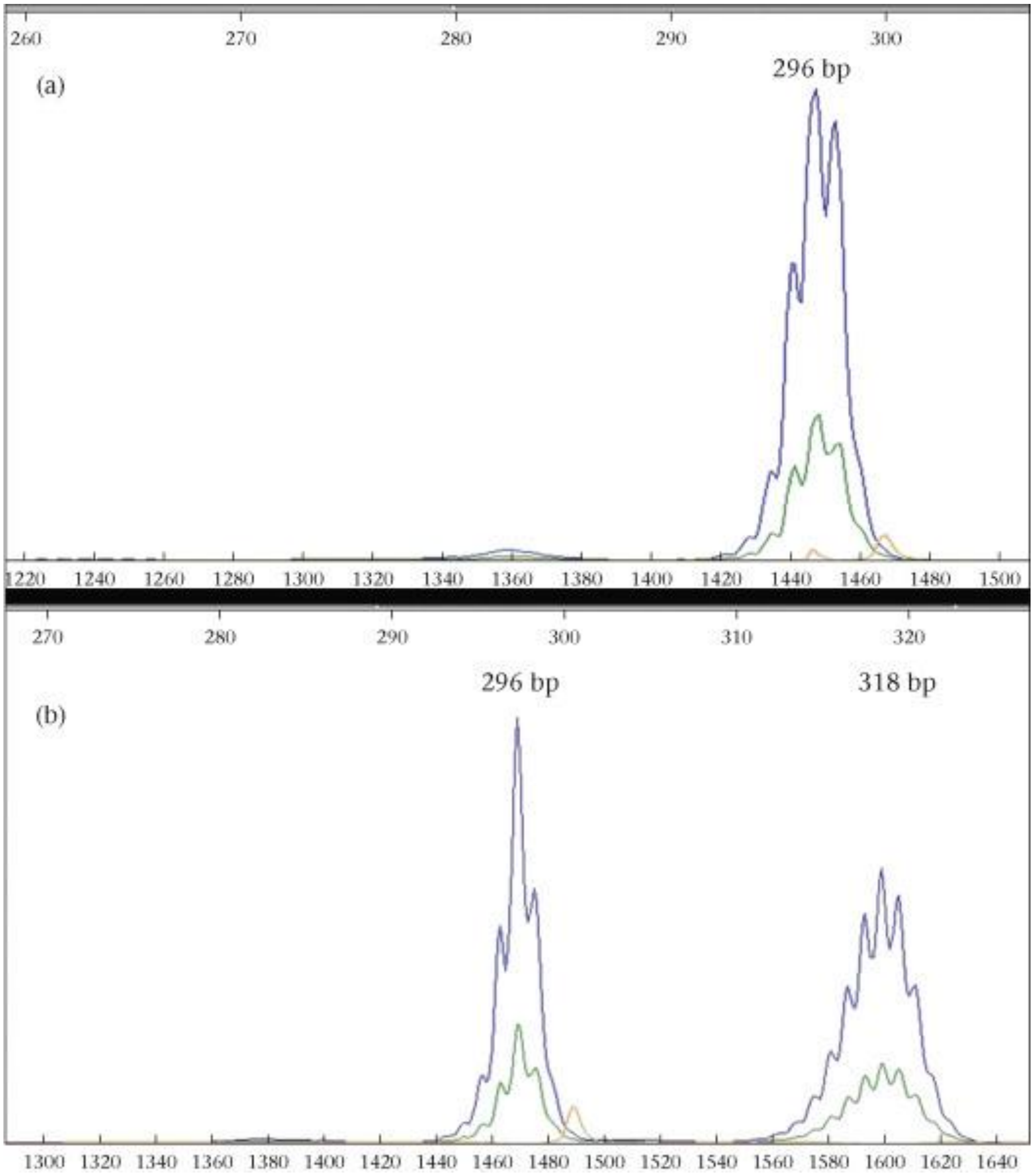

Figure 3. Eleonora's falcon sex ratio at fledging in relation to (a) hatching date and (b) hatching order.

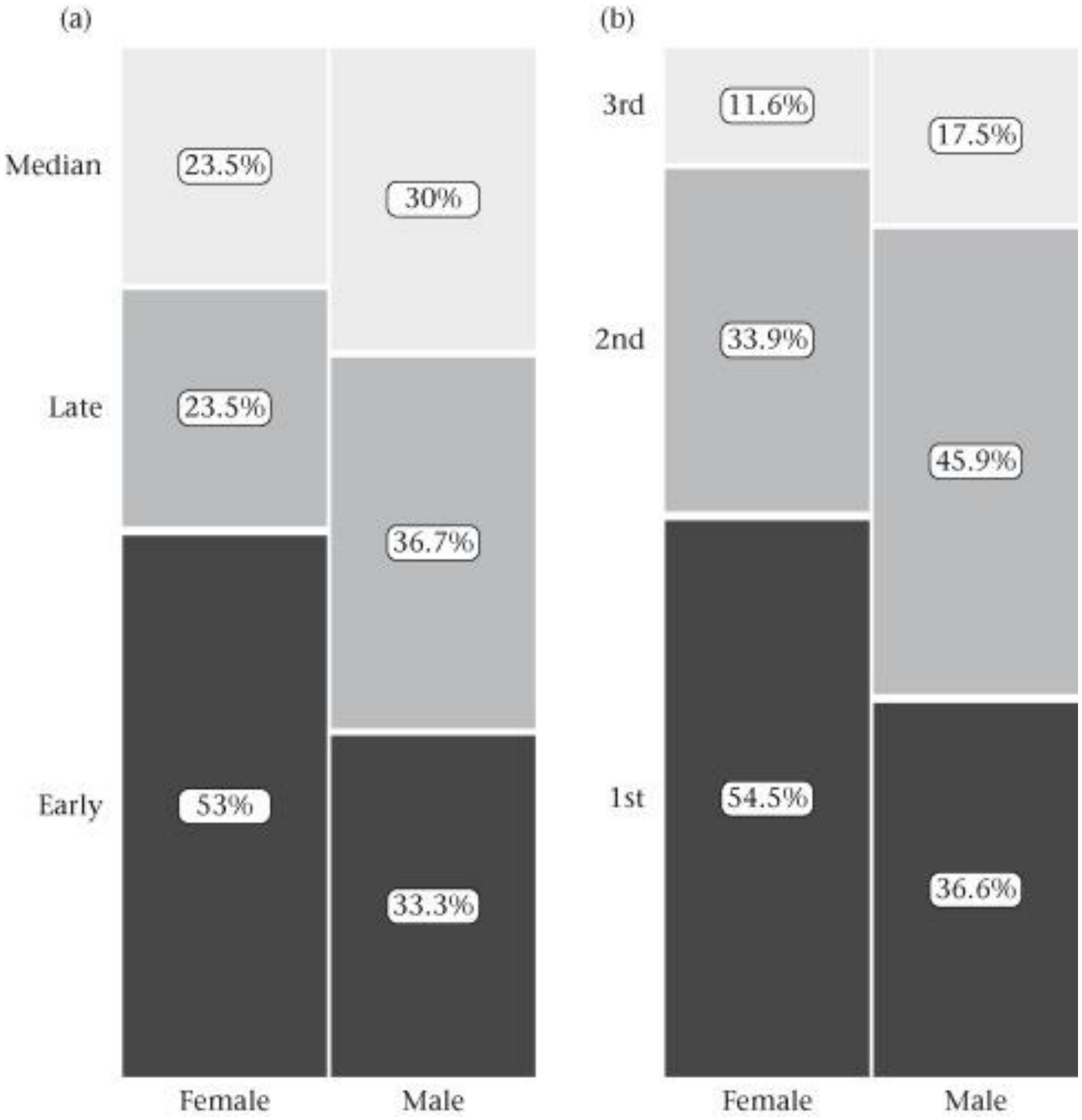

Figure 4. (a) Percentage of female fledglings in relation to environmental conditions estimated indirectly by the number of fledglings/successful pair per year (<1.8: ‘bad’ years'; ≥1.8: ‘good years’). The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range. (b) Linear regression model with 95% confidence intervals (shaded area) between the percentage of female fledglings and a population density index measured as the mean nearest-neighbour distance between nests per year.

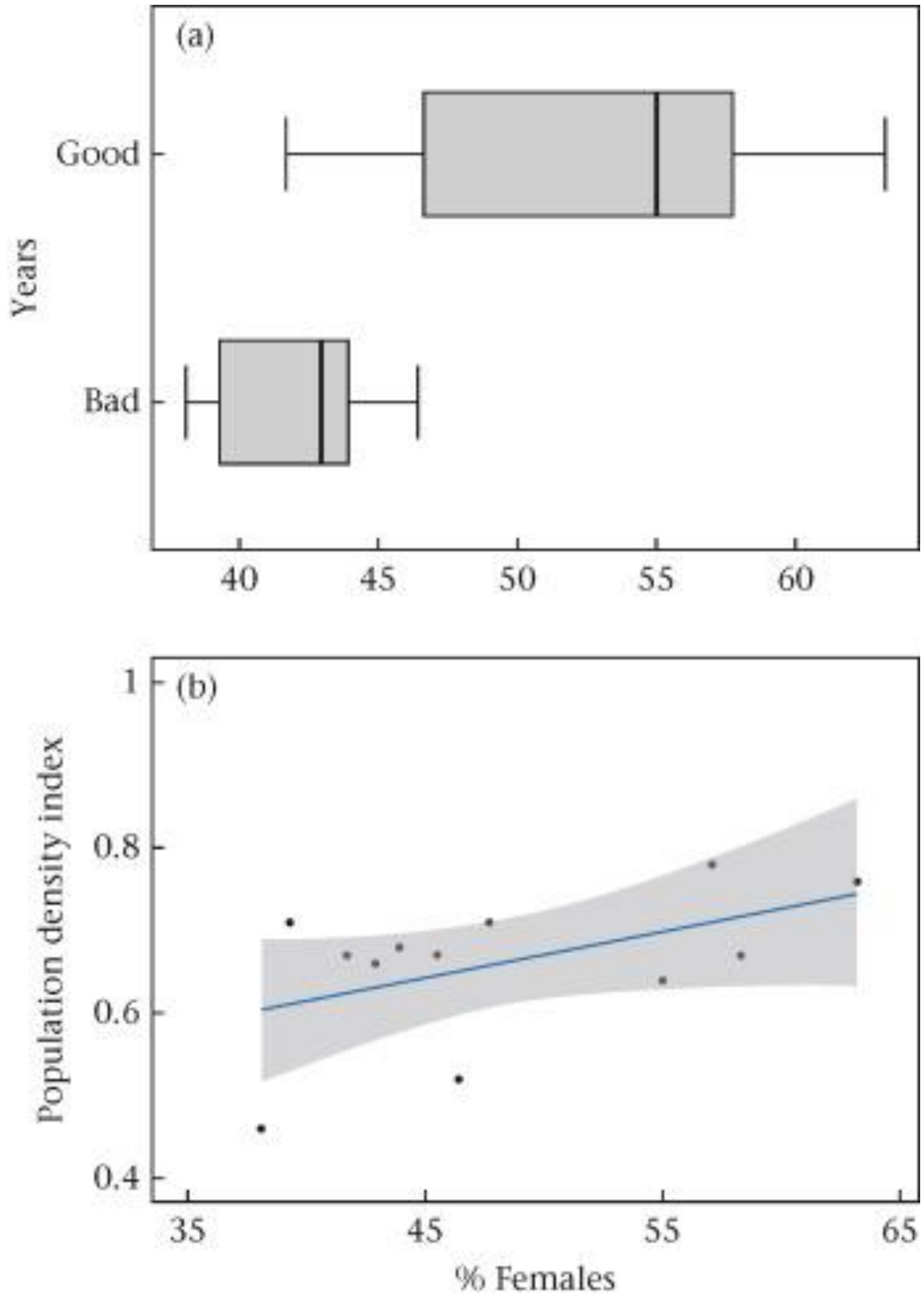

Figure 5. Body condition (i.e. standardized residuals of a linear regression of body weight on wing length) of fledglings in relation to (a) hatching period and (b) hatching order. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the circles are outliers. Statistical comparisons were made by Kruskal–Wallis and post hoc pairwise comparison tests.

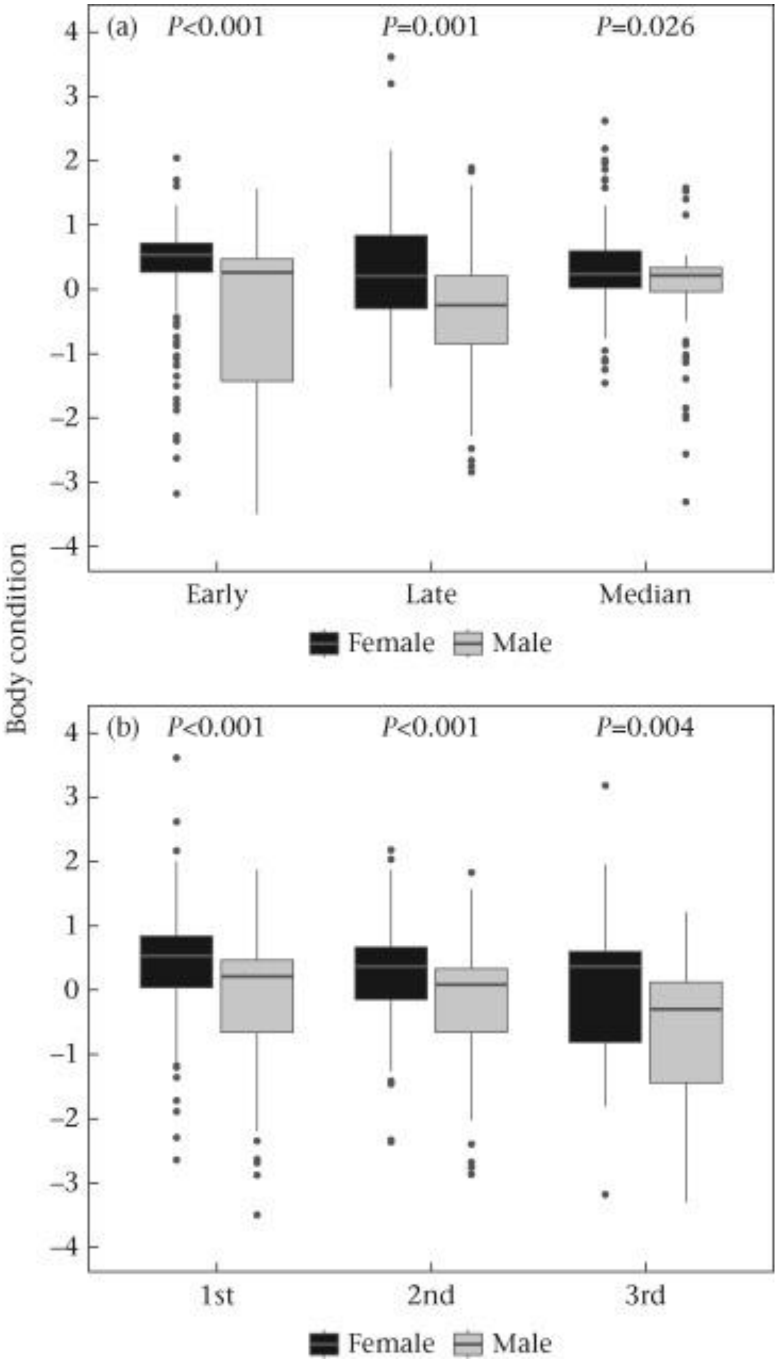

Figure 6. Body condition (i.e. square-root transformed standardized residuals of body weight on wing length) of (a) male and (b) female) fledglings in relation to the interactive effect of age and hatching order (predicted values and 68.3% confidence intervals). Data are from clutches with zero losses until fledging.