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Breeding biology and inheritance of a color pattern in a monomorphic bird

A dissertation submitted in partial satisfaction of the requirement for the degree

Doctor of Philosophy

in

Biology

by

Alexa Lee-Lang Bontrager

Committee in charge:

Professor Joshua Kohn, Chair
Professor Trevor Price, Co-chair
Professor James Goodson
Professor Philip Hastings
Professor Karen Marchetti

2006

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University of California, San Diego

2006

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Bontrager, A.L. and T. D. Price (submitted). Genetic correlation between the sexes in the color pattern of a sexually monomorphic bird. *Evolution*.

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ABSTRACT OF THE DISSERTATION

Breeding biology and inheritance of a color pattern in a monomorphic bird

by

Alexa Lee-Lang Bontrager

Doctor of Philosophy in Biology

University of California, San Diego, 2006

Professor Joshua Kohn, Chair

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A number of factors influence the expression of an individual's sexually selected traits. These include genes, environmental factors, parental effects, and the individual's condition. Parental effects can also affect offspring condition. This dissertation considers factors influencing offspring condition, and how genes and parental effects contribute to the expression of the sexually selected wing-bar *Phylloscopus humei*, a monomorphic species. Males use their wing-bars in interactions determining territory size, but other forms of selection on the wing-bar have not been identified. I studied a breeding population of *P. humei* in the Manali Sanctuary, Manali, Himachal Pradesh, India breeding along an elevational gradient.

In Chapter I, I use this elevational gradient to gain insight into factors that might explain nestling condition. The hatch date of nestlings has been commonly found to be important in their survival and fitness, with earlier-hatching birds faring better than those hatching later in the season. It is unclear whether this effect results as a direct consequence of the actual date of hatching or via parental transmission of fitness to offspring (with higher condition birds breeding earlier). I found that chicks hatching at higher elevations did so later in the season and were in poorer condition than were chicks hatching earlier at low elevation. There was no detectable compensation for late breeding at higher elevations, suggesting that parents pass their condition to their offspring.

The following two chapters examine possible influences on monomorphism, focusing on the wing-bar. In Chapter II, I assess extra-pair paternity in the species and how it might affect selection on the wing-bar. Using newly isolated microsatellite primers, I assessed parentage in this population as well as in a handful of families sampled in Naran, Pakistan. The rate of extra-pair paternity was high and similar across years and in two populations—35% overall in Manali and 32% in Naran. There was no association of wing-bar size, either in adult males or in offspring, with extra-pair paternity, and it appears that the high levels of extra-pair paternity may be a result of male harassment of females owing to the high density and breeding synchrony of the species.

Chapter III considers another possible influence on monomorphism in *P. humei*—the inheritance of wing-bar size. Using parent-offspring regressions across sexes, this chapter determines that the wing-bar's presence in females is a result of a genetic correlation between the sexes. This genetic correlation is sufficiently strong that

dimorphism is not expected to develop. However, there is a difference in the magnitude of heritability of wing-bar size between mothers and their sons and daughters. This may be the result of a sex-specific maternal effect acting on inheritance of the size of the wing-bar. Additionally, based on observations of female behavior, it is likely that the wing-bar may serve them a function, though it has not yet been determined.

CHAPTER I

Causes of the seasonal decline in offspring fitness: evidence from elevational variation

Abstract

In many bird species, nestling condition declines with increasing hatch date. This is attributed to effects of either parental quality (condition is transmitted from parent to offspring, and higher quality parents breed earlier in the season) or to environmental conditions experienced at different times (i.e., time of breeding). Here we take advantage of elevational variation (individuals at higher elevation breed later in the season), to show how the effects of parental condition and time of breeding can be partitioned. We predict that a direct effect of lay date on offspring survival will produce higher elevation nestlings in higher condition to compensate for the advantages of early breeding at lower elevation. Alternatively, if the correlation between nestling hatch date and condition arises entirely through parental condition, nestlings at higher elevation should be in similar or lower condition to those at lower elevation. In a study of Hume's warbler, *Phylloscopus humei*, in the western Himalayas, we find that nestling condition declines with elevation. The simplest explanation is that the seasonal and elevational decline in offspring condition is largely a result of parental quality, with low quality parents segregated to higher elevations. There is no evidence for direct selection on breeding date associated with nestling survival. We suggest that for this species, effects of early breeding on parental survival and future reproduction may be one critical factor determining early breeding in high quality individuals.

Introduction

In many species, individuals that breed earlier in the season have higher numbers of surviving young than those that breed later (Meijer et al. 1990; Sheldon et al. 2003). Given such strong selection pressure to breed early, the reason that the population as a whole does not evolve to breed at an earlier laying date has been widely discussed and is still considered unresolved (Sheldon et al. 2003). Van Noordwijk et al. (1995) describe a number of possible explanations, two of which are most commonly considered. In the first, an offspring's fitness is not directly affected by the date on which it hatches. Instead, adults in high condition breed early and impart their condition to their offspring, which then have higher survival as a result. Although high condition individuals both breed early and have more surviving offspring, average condition cannot increase across generations, so average breeding date will not either (Price et al. 1988; Cooke et al. 1990). The question of why adults in high condition should breed early is either left unaddressed (Price et al. 1988) or explained in terms of their own survival and reproduction (Drent and Daan 1980).

In the second explanation, the date on which offspring hatch does directly affect their fitness, and earlier-hatched offspring have higher fitness. For example, more food may be available earlier in the season, or earlier-hatching offspring have more time to gain foraging skills for the upcoming winter. Although lay date is heritable (Price et al. 1988, Sheldon et al. 2003, Charmantier et al. 2005), it does not evolve because of selection acting at other stages of either adult or offspring life cycle or because the response represents a recent change in the environment, e.g., because of global warming.

Much recent empirical effort has been directed at assessing the relative contributions of parental condition and lay date directly through experimental manipulation (e.g., Verhulst et al. 1995; Wardrop and Ydenberg 2003; Arnold et al. 2004). In particular, lay and hatch dates can be altered by shifting clutches among parents or into and out of a refrigerator, and condition altered by feeding experiments. These results show that parental condition usually does affect offspring condition, but it is very difficult to manipulate parental condition and lay date independently (Sheldon et al. 2003).

In this paper, we use an alternative non-experimental approach to assess the relative contributions of parental condition and lay date to offspring fitness by making use of the fact that, within species, lay date is correlated with elevation along altitudinal gradients; individuals breeding at high elevations breed later (Table 1; Slagsvold 1976). If lay date directly influences offspring survival, individuals breeding at low elevations should have an advantage through this component of fitness. Thus, if lay date does have a direct effect, we should be able to detect a countervailing selection pressure favoring later breeding at higher elevation.

Our purpose here is twofold. First, we formalize the two alternative models using path diagrams and show how they can be applied to the study of elevational variation. Second, we investigate this elevational variation in the reproductive parameters of a small warbler, *Phylloscopus humei*, breeding in the northwest Himalayas. We use correlates of nestling condition with elevation to assess the alternative models for the decline in offspring fitness with lay date.

Models of the decline of offspring fitness with lay date

A general principle of life-history theory is that, if costs of investment into different life-history stages accumulate more-or-less additively, but benefits accumulate more-or-less multiplicatively, individuals with additional energy to invest should apportion that energy amongst all traits, and not in just one (e.g., Rowe et al. 1994; Appendix). Thus, individuals in low condition should invest less into both their own maintenance and their reproduction than individuals in high condition. Further, with respect to reproduction, individuals in low condition should invest less in both offspring quality (i.e., the food and resources they provide to their offspring) and in offspring number, than should individuals in high condition.

Drent and Daan (1980), Daan et al. (1990), and Rowe et al. (1994) used these principles to explain why individuals in low condition should breed later in the season than individuals in high condition. A critical assumption of their models is that there is a direct effect of lay date on offspring fitness. In this case, it pays parents to breed early, so, assuming equilibrium, some counterbalancing force is required. The second component of the models is thus a cost to early breeding. Rowe et al. (1994) suggested that one cost is reduced fecundity: early breeding females have had less time to accumulate resources to invest in their offspring than late-breeding females. Daan et al. (1990), in their studies of the kestrel, *Falco tinnunculus*, assumed that costs could lie anywhere in the life-history, including reduced survival and future reproduction. Given both costs to early breeding (on an individual's fitness), as well as benefits to early breeding (on offspring fitness), individuals in higher condition are expected to invest

more into both breeding earlier in the season and into their offspring than individuals in lower condition (Rowe et al. 1994). This is illustrated in the path diagram of Figure 1A and in Figure 2.

The model of Rowe et al. (1994) is based on the assumption that the environment steadily deteriorates through the season, with direct effects on offspring fitness. However, an alternative is that lay date has no directional effect on offspring fitness. Instead, costs and benefits of breeding early accrue to the parents in terms of their own prospects of future reproduction. As before, parents in high condition breed earlier than those in low condition and differentially impart their condition to their offspring. Because the correlation between offspring fitness and lay date is entirely through condition, which cannot evolve, no counterbalancing selection is required at equilibrium (Price et al. 1988). The result is a simpler path model (Figure 1B).

Elevation

We suggest that the question of whether there is a direct path from lay date to offspring fitness (e.g., Figure 1A), or not (Figure 1B), can be assessed using information on offspring fitness in association with elevation. An earlier onset of spring-like conditions means that individuals breeding at lower elevations breed earlier. Predictions differ depending on whether advantages to early breeding accrue within the nest or post-fledging (see below). The predictions are as follows:

- 1) No direct path: Parents of high condition breed early and impart their condition to their offspring. Parents of lower condition move to higher

elevation where they breed later in association with later appearance of resources. We predict nestling condition to decrease with elevation.

- 2) Direct path: There is directional selection on lay date, but this can arise in two ways.
 - a) First, Perrins (1970) suggested that the direct effect of lay date arises because the amount of food available for parents to adequately provision offspring declines through the season. This should be reflected in offspring condition. Because the flush of resources occurs later at higher elevations, the direct effect of lay date acts within and not between elevations. We predict that offspring condition is similar across elevations (Figure 3A).
 - b) The direct selection on lay date arises as a result of post-fledging juvenile experience—late-hatched young are younger and likely to be less experienced than early-hatched young. This is a between-elevation effect because young from different elevations should regularly come into contact and compete with each other. In this case, the direct effect of lay date accumulates across elevations. We predict that to compensate, offspring condition is higher at higher elevations (Figure 3B).

We now present an empirical study designed to test these alternatives.

Methods

Study system

Phylloscopus humei is a small (6g), insectivorous, passerine that breeds abundantly through the western Himalaya (Price and Jamdar 1991a). We studied a population in Manali Sanctuary, Himachal Pradesh, India (at 32° 14'N, 77° 7'E) in May-June 2001, 2003, and 2004. *P. humei* is a ground nester, commonly breeding in association with birch, *Betula utilis*. Nest building cannot commence until after snowmelt, but in years of heavy snow building begins as soon as the snow disappears (Price and Jamdar 1991a). Females build nests and incubate clutches alone. They lay one egg per day on successive days, beginning incubation after the clutch is complete. Clutch size at Manali was generally 4 eggs (77%), but during our study, 8% of all clutches contained 3 eggs, and 14% contained 5 eggs. Incubation time was approximately 14 days in length. Both parents feed nestlings, and juveniles fledge at approximately 12 days of age. Nest sites were positioned from 3100 – 3500 m. above sea level.

Nests were typically found in the building stage or when parents were feeding chicks. In 2001, elevation of nests was noted using an altimeter (i.e., these measurements were based on barometric pressure, and, depending on weather conditions, readings could differ for the same location). In 2003 and 2004, nest elevations were determined using GPS (Garmin eTrex). Twenty nest elevations measured in 2001 by altimeter were compared to those obtained the following year by GPS, and the average difference

between the two sets of measurements (55 m) was added to each nest elevation that was obtained in 2001.

Egg volume has been used as a measure of female quality (Williams 1994), and in some birds, there are differences in egg volume with elevation (Table 1). In 2003, the length and breadth of eggs from complete clutches were measured with calipers. Clutches were considered complete when a new egg was not laid the following day or if the female was known to be incubating the clutch. Found nests were checked infrequently so as not to attract predators, but where possible, the date of the first egg laid (first egg date) was noted. Some nests (52% in 2001, 51% in 2003, 24% in 2004) were covered with wire baskets to protect them from avian predators.

The age of nestlings can be assessed via visual inspection. We banded and measured nestlings (see below) at approximately ten days of age. In this study, we assume that banding date is strongly correlated with lay date. In fact, in a previous study in Overa, Kashmir, Price and Jamdar (1991a) showed that later breeding individuals had shorter incubation times. This effect seems to be present in our data, based on eight nests we followed from laying of the first egg to banding of nestlings. Among these eight nests, the regression of days in the incubation and nestling period against first egg date was $b = -0.12$, implying that for every eight days later in the season that the first egg was laid, the incubation and nestling period was one day shorter ($r = -0.61$; $P = 0.11$). The effect is small; the mean number of days from laying of the first egg to banding was 28 days, with a predicted difference of 1.5 days between the extremes in lay date. We used this information to estimate first egg date for clutches (whose eggs were measured) for which it was not directly observed, but from which we banded nestlings.

In some analyses, we segregate nestlings by sex; the sex of nestlings was determined from blood samples, using a molecular technique (Bontrager et al. unpublished manuscript). Nestlings were banded and weighed (using a spring scale) and their tarsus length measured (to the last complete scale; Svensson 1992).

We use tarsus length as a measure of condition. In other studies, tarsus length has been shown to be a strong correlate of nestling survival (Ringsby et al. 1998; Alatalo et al. 1990; Kruuk et al. 2001). Higher growth rate and larger size in juveniles is often associated with increased survival probability, both to fledging and to breeding (Perrins 1965; Tinbergen and Boerlijst 1990; Magrath 1991). We present measures based on body weight, but the patterns are less strong.

Statistical analyses

Because juveniles in a given nest are genetically similar and share a common nest environment, we used brood averages for measured traits. Statistical tests were conducted using JMP IN (SAS Institute, Cary, NC). Mean values across years for continuous variables (banding day, elevation, chick tarsus length, and chick weight) were compared using ANOVA. Clutch size and fledging numbers were compared across years using Chi-square contingency tables. A general linear model was used to control for effects of year in investigating the effects of elevation and banding date on condition. Two-tailed P-values are reported throughout.

Results

Among year variation

We first tested for differences between years in order to account for such differences in future analyses. Statistics for each year of the study for banding day, nest elevation, clutch size, number of fledglings, chick tarsus length, and chick weight are given in Table 2. Birds bred significantly earlier in 2001 than in the other two years. The number of chicks fledged in 2004 was lower than in 2001 and 2003. Surviving nestlings were also in lower condition (i.e., had shorter tarsi and lower body weight) in 2004 than the other years (Table 2). Because of differences between years, we included year as a factor in the statistical model.

The time of day at which a nestling is measured influences its weight (effect test for weight, controlling for year, $b = 0.049 \pm .009$ g/h; $F_{1,86} = 28.03$; $P < 0.0001$) but not its tarsus length (effect test for tarsus, controlling for year, $b = 0.011 \pm .0010$ mm/h; $F_{1,85} = 1.10$; $P < 0.30$). Residuals of both tarsus length and weight, each regressed on both year and time of measurement were used to control for those two factors. Tarsus length and weight are correlated ($r = 0.34$; $P = 0.0012$; $N = 89$).

Correlates of banding date with tarsus length

Birds breeding at higher elevations bred later in the season than did those at lower elevations (Figure 4A; Table 3). Chick tarsus length decreased with increasing

elevation and with later laying date (Figure 4B, C; Table 3). These results were maintained in a multiple regression analysis: tarsus length declines with both elevation and, if four outliers (see Figure 3B) are removed, banding date when both are included in the model (Table 3). When these nests are left in, in the multiple regression, elevation remains a significant factor, while the P value of banding date is 0.09.

The results were similar when the data were reanalyzed after separating the nestlings by sex: among females an increase in elevation ($F_{1,68} = 4.73$, $P = 0.03$) and banding date ($F_{1,68} = 2.64$, $P = 0.11$) were both associated with a decline in tarsus length when these factors were included in a multiple regression. A similar result was found for males (elevation $F_{1,63} = 2.07$, $P = 0.16$; banding date: $F_{1,63} = 4.07$, $P = 0.05$). In conclusion, both elevation and banding date are independently correlated with nestling tarsus length,

Egg volume

Eggs were measured in 2003, and following Marchetti (2000), egg volume was estimated as proportional to length x (breadth)². One outlier was eliminated from the analysis. There was no association between egg volume and elevation ($b = -0.02 \text{ mm}^3/\text{meter} \pm 0.25$; $r^2 = 0.0002$; $F_{1,32} = 0.01$, $P = 0.93$). There was also no relationship between mean egg volume and first egg date ($b = 6.53 \pm 6.69$; $r^2 = 0.05$, $F_{1,18} = 5.2$, $P = 0.34$). This result differs from the finding of Marchetti (2000) studying the same population in 1994. She found egg volume decreased with lay date ($b = -10 \text{ mm}^3/\text{day}$, $N = 14$ clutches, $P = 0.07$).

Discussion

A decline of chick tarsus length with lay date has been previously reported from another population of *P. humei* breeding at Overa, Kashmir (Price 1991), although elevation was not measured in that study. In the population of *P. humei* that we studied (henceforth, the Manali population), the span of lay dates is over only 14 to 18 days (depending on the year), yet we found that measures of nestling condition decreased with both lay date and elevation. Based on the alternative predictions developed in the introduction, we suggest that this is largely a consequence of lower condition adults breeding at later dates and settling to breed at higher elevation. We have been unable to directly measure adult condition, but instead, focus on the condition of their offspring, which is much more variable among individuals, being reflected in differential growth.

In other studies, declines of nestling condition with lay date have been attributed to a deteriorating environment, i.e., reduced food supply later in the season, rather than parental condition. This has been particularly applied to hole nesting *Parus* species and related to declines in their food supply (Perrins 1970; Perrins and McCleery 1989; Van Noordwijk et al. 1995). In our study, as in a study of the kestrel (Daan et al. 1990), it is not clear that food supplies do in fact decrease over the spread of nesting dates. In Overa in 1986 and 1987, caterpillar abundance actually increased throughout the nestling period of *P. humei* (Katti and Price 2003). In addition, most other species of warblers at these elevations breed later than *P. humei*, suggesting that arthropod abundance remains high (Price and Jamdar 1991b).

The possibility remains that early-hatching chicks have an advantage because they are older; we expect some form of compensation for this in later-hatching chicks. Although we reject higher condition as a compensatory factor, there may be possibilities, some of which we did not measure. For example, we fail to find an increase in clutch size or egg with increasing elevation and hence later lay date. A tendency for incubation period to be shorter later in the season (an association shown to be significant in the Overa study; Price and Jamdar 1991a) should reduce the chances of predation later in the season and could compensate for later hatching chicks. However, we could find no evidence for an effect of lay date on nest predation (logistic regression controlling for year $\chi^2_{32} = 32.9$; $P = 0.43$, $N = 35$ nests), nor was there any evidence that predation correlates with lay date in Overa (Price and Jamdar 1991a). In summary, our failure to find any sort of compensatory benefits to young hatching at higher elevation leads us to conclude that the simplest explanation for the correlation of lay date with offspring fitness is that it is driven mostly through parental condition.

Although nestling condition has not previously been measured, our findings are consistent with those from other studies. Of 45 species surveyed, Krementz and Handford (1984) report that 26 (58%) show a decrease in clutch size, while 11 (24% show an increase) with elevation. There is no effect in 8 (18%). Table 1 shows the findings of more recent studies. Although 4 of 10 studies report an increase in clutch or egg size, 5 studies report a decrease in one such measure with elevation. When the results of the studies listed in Table 1 are combined with those summarized in Krementz and Handford (1984), 31 of 46 studies showing an effect of elevation on a measure of reproductive output show a decrease ($\chi^2_1 = 5.57$; $P < 0.05$). A decrease in clutch size with

increased elevation has been reported in comparisons of populations or closely related species at different elevations as well, although high elevation parents may compensate by investing more energy in parental care (Badyaev and Ghalambor 2001). It is thus not apparent that benefits to breeding at high elevation should act through clutch or egg size.

Van Noordwijk et al (1995), Merilä et al (2001b), and Sheldon et al. (2003) list several alternative explanations for why lay date appears to be under directional selection. One possibility is that directional selection on lay date is a recent phenomenon resulting from change in environmental conditions, and the population is not at equilibrium. While it may seem unlikely that this could apply to so many examples, in several cases, there has been supporting evidence (e.g., reduced fledging success in later years of the studies) that the environment is indeed currently deteriorating (Cooke et al. 1990; Merilä et al. 2001a; Garant et al. 2004). There is also evidence that the mean genotype is increasing (Merilä et al. 2001a; Garant et al. 2004).

In our study, it is possible that environmental (e.g., climatic) variables have in fact recently changed, and the negative association of condition with elevation represents a recent, transient, phenomenon. In earlier years at Manali (1994-1996; Marchetti 2000) snow-melt was about one month later than it was during our study (2001-2004), perhaps attributable to global warming (*personal observations*). Associated with this, chicks in the earlier years were raised during typically warm periods with occasional storms, whereas conditions more recently have been often misty and wet. In 2004, a particularly wet three days during the middle of the fledgling period was associated with much abandonment of nests at higher elevation (of 38 nests discovered over the season that reached the nestling stage and were not predated, 9 nests containing a total of 25 young

were abandoned). That there are real differences between the earlier years and later ones is suggested by Marchetti's (2000) finding that egg size was negatively correlated with lay date (elevation was not measured), whereas in our study, we were unable to find such a correlation. If climatic changes are resulting in direct selection to breed at lower elevation, they place an interesting twist on the general effects of global warming which seem to be generally increasing latitudinal and elevational range limits and advancing lay dates (reviewed in Crick 2004).

While changing environments may contribute to some examples of persistent directional selection on lay date, including that in our study, we see this as unlikely to be the general explanation because the phenomenon is so widespread. One argument against the primary role for condition dependence is that the genetic (breeding) values for the trait in question can be shown to have increased (Merilä et al. 1991a; Garant et al. 1994). However, if the trait is correlated with condition, and condition itself has a substantial additive genetic component (Rowe and Houle 1996), even though breeding values increase, the phenotypic values cannot. Thus, the increase in breeding values of the trait may reflect the increase in breeding values for condition, which is correlated with the trait. Genetic variance in condition is likely maintained by recurrent deleterious mutations and fluctuating environments (such as host-parasite co-evolution; Cooke et al. 1990; Burt 1995; Rowe and Houle 1996).

Our results have implications for the general applicability of the model of Drent and Daan (1980) and Rowe et al. (1994) who argued that high quality individuals breed early because of the favorable effects early breeding has on offspring survival. If there is no direct selection on lay date via offspring survival, these models do not apply. It is

possible that in *P. humei*, the main selection pressure favoring early breeding is through adult survival: high quality individuals are able to return to breeding grounds more quickly, complete breeding more quickly, go through the post-nuptial molt sooner, and thereby establish high quality territories in their winter quarters (individual birds hold territories through the winter in the northern part of India (Gross and Price 2000). *P. humei* is a single brooded migratory species that breeds earlier than other warbler species at similar elevations in the western Himalayas, and the results may not generalize to these other species nor to resident species such as tits. They point to the likelihood that many different features of the life history are likely to favor earlier breeding by high quality individuals.

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Appendix: Accumulation of costs and benefits

Here we show that if costs accrue additively, but benefits multiplicatively (e.g., probability of adult survival $\times$ number of offspring, or number of eggs $\times$ probability of nestling survival), then increased investment into both stages of a life history is expected. Assume fitness of an individual across the two stages of the life history is given by x and y respectively, so total fitness, F , is xy . Now assume that individuals have an amount of condition, c , that can be invested into the two traits, $c = kx + y$, where the constant k scales the relative amount invested in x and y . Using this latter expression to eliminate the variable y , we write individual fitness as:

$$F = kx(c - kx)$$

Differentiating this expression with respect to k and setting it equal to 0, we obtain an expression for optimal investment into x :

$$k_{(opt)} = (c/x)/2 = y/x$$

If x and y have equal effects on fitness, exactly half of any additional investment should be apportioned to each stage, otherwise more is invested in the trait with lower fitness, to maximize the product. This applies to all models where costs are not multiplicative and is the general principle underlying the model of (Rowe et al. 1994) for a seasonal decline in parental investment in the face of a decline in offspring survival.

Table I.1. Effects of elevation on reproductive traits in bird species. These are studies not reported in Krementz and Handford 1984.

Species	Description	Elevation	Effect of increased elevation	Reference
<i>Cinclus mexicanus</i>	1 watershed	28 - 800m	no effect on annual productivity	Morrissey 2004
<i>Corvus corax</i>	1 island	0 - 1200m	later lay date fewer young fledged	Nogales 1995
<i>Ficedula hypoleuca</i>	103 areas	5 - 1700m	later lay date smaller clutches	Jarvinen 1989
<i>Ficedula hypoleuca</i>	99 areas	5 - 1900m	later lay date smaller clutches	Sanz 1997
<i>Parus caeruleus</i>	87 populations	4 - 1650m	later lay date smaller clutches	Fargallo 2004
<i>Parus major</i>	5 sites	30 - 1100m	later lay date larger clutches	Beldal et al. 1998

Table I.2. Reproductive parameters for each of the three years of study.

	2001			2003			2004		
	Mean $\pm$ s.d.	N		Mean $\pm$ s.d.	N		Mean $\pm$ s.d.	N	
Banding day	166 $\pm$ 4.3 ^a	29		174 $\pm$ 0.7	32		172 $\pm$ 2.2	25	¹ F _{2,83} = 36.9 < 0.0001
Elevation	3311 $\pm$ 83.3 ^b	29		3358 $\pm$ 70.4	30		3381 $\pm$ 77.3 ^b	28	¹ F _{2,84} = 6.15 0.0032
*Clutch size	3.9 $\pm$ 0.7	37		4.2 $\pm$ 0.5	45		3.9 $\pm$ 0.5	22	
No. fledglings	3.2 $\pm$ 0.9	29		3.5 $\pm$ 1.1	32		2.7 $\pm$ 0.8 ^a	26	χ^2_4 = 17.0 0.002
Chick tarsus	17.6 $\pm$ 0.4	29		17.4 $\pm$ 0.7	32		16.7 $\pm$ 0.7 ^a	27	¹ F _{2,85} = 15.7 < 0.0001
Chick weight	7.0 $\pm$ 0.6	29		6.9 $\pm$ 0.5	32		6.2 $\pm$ 0.7 ^a	27	¹ F _{2,84} = 15.7 < 0.0001

¹F or Chi-square statistic testing for differences among years, with associated P-value.

Replication was always based on clutch; N = number of clutches.

* No test was possible because the majority of clutches contained 4 eggs.

^a Value is significantly different from values in each of the other two years (P < 0.05; Tukey test).

^b Two values are significantly different from each other (P < 0.05; Tukey test).

Table I.3. Linear regressions of tarsus and weight on banding date and elevation.

Factor	Tarsus b $\pm$ s.e.	Tarsus b $\pm$ s.e.	Tarsus b $\pm$ s.e.	Weight b $\pm$ s.e.
Day	-0.046 $\pm$ 0.017**	-	-0.030 $\pm$ 0.017	-0.0089 $\pm$ 0.0186
Elevation	-	-0.0026 $\pm$ 0.0008**	-0.0022 $\pm$ 0.0008**	-0.0011 $\pm$ 0.0009
F-statistic (model)	F _{3,84} = 13.78**	F _{3,82} = 14.83**	F _{4,81} = 12.12**	F _{4,81} = 7.71**

Year is included in the model. Missing entries indicate that factor was not included. *P < 0.05; **P < 0.01. These models were run with the inclusion of four outliers. Exclusion of the outliers does not change significance levels of any of the factors except in the third column, where, for elevation, P = 0.03 and for day, P = 0.01.

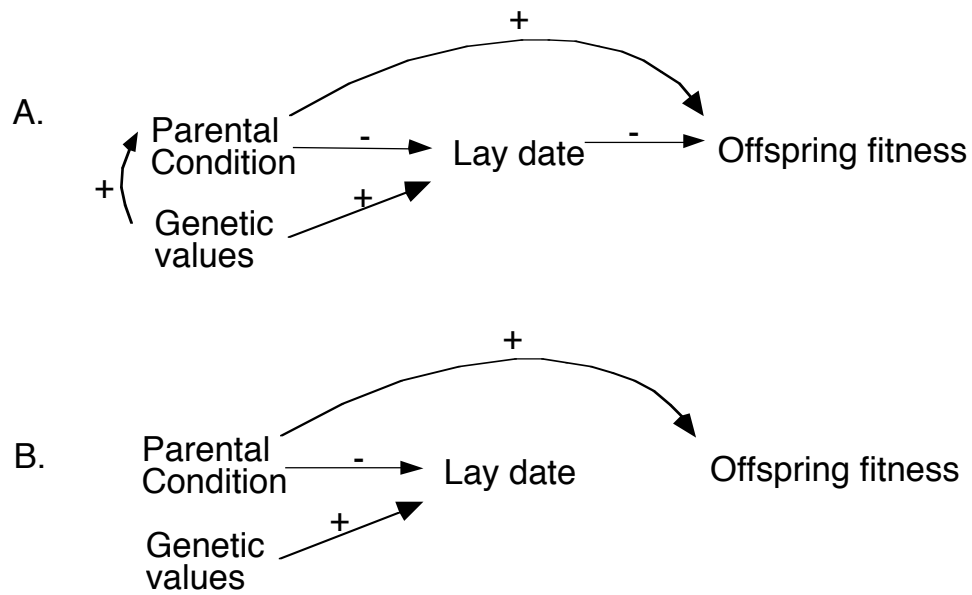


Figure I.1. Path models that lead to a persistent correlation of offspring fitness with lay date. Only non-zero paths are shown. A: The model of Rowe et al. (1994). In this model, offspring fitness is directly affected by lay date. This is balanced by higher investment by parents later in the season. B: A second model where offspring fitness is not directly affected by lay date (from Price et al. 1988).

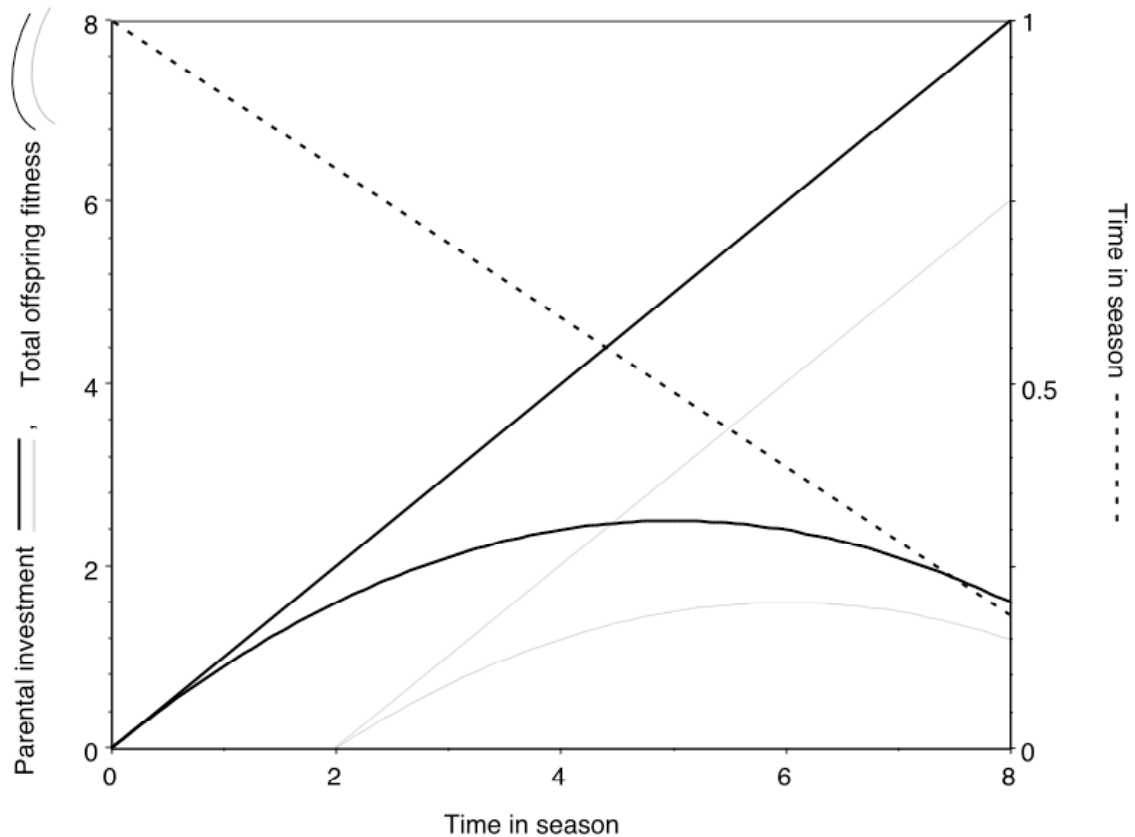


Figure 2. The model of Drent and Daan (1980) and Rowe et al. (1994) drawn to illustrate stabilizing selection on genetic values for laying date. The dashed line indicates decline in offspring survival through the season as a direct result of lay date (T) (the curve is $Y = 1 - T/10$). The solid linear curves indicate accumulation of condition (= parental investment) with time for high (dark; $Y = T$) and low (light; $Y = T - 2$) quality females. The curved lines give the product of parental investment and offspring survival (i.e. fitness) for each of low and high condition individuals: the optima are at $T = 5$ (high) and $T = 6$ (low). Note that for both high and low condition, individuals with genetic values to breed early invest less than is optimal in their clutch.

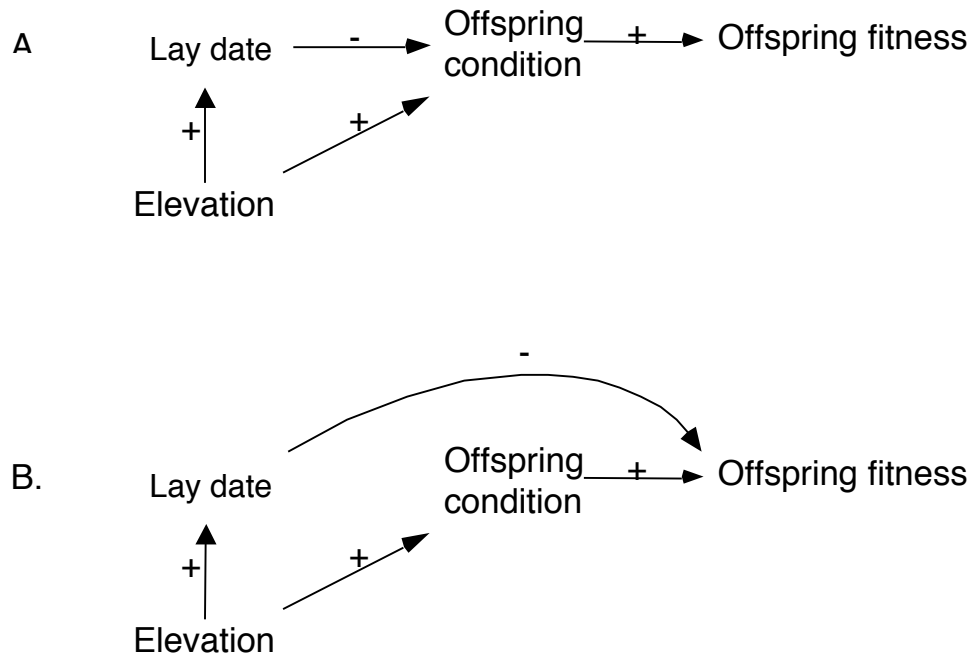


Figure 3: Models of the way higher offspring condition at higher elevation can balance costs of later breeding. Only non-zero paths are shown. Given direct selection on lay date, the correlation of offspring condition and elevation depends on whether the direct effect of lay date acts through parental provisioning in the nest or post-fledging, e.g., as a result of older age in early-hatched chicks. A: Parental provisioning declines with lay date but increases with elevation. These effects cancel, so there is no association of offspring condition with elevation. B: The direct effect acts on chicks post-fledging. In this case, the correlation of condition with elevation is positive.

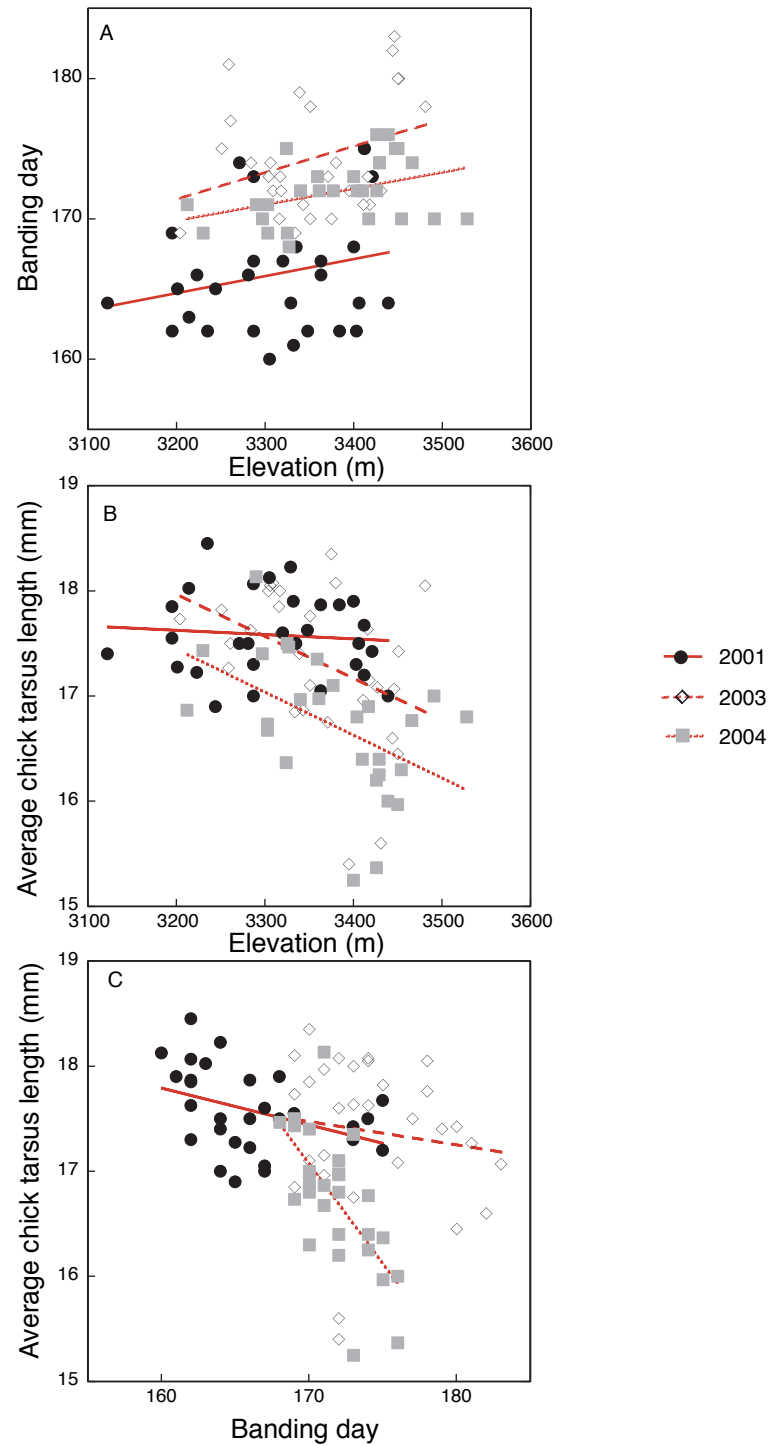


Figure I.4. Regressions of tarsus length, banding date, and elevation by year. A: Regression of banding date on nest elevation. 2001: $y = 0.012x + 125.7$; 2003: $y = 0.019x + 111.1$; 2004: $y = 0.011x + 133.2$. B: Regression of average (per nest) chick tarsus length on nest elevation. 2001: $y = -0.000x + 18.9$; 2003: $y = -0.004x + 30.7$; 2004: $y = -0.004x + 30.5$. C: Regression of average (per nest) chick tarsus length on banding day. 2001: $y = -0.035x + 23.397$; 2003: $y = -0.022x + 21.252$; 2004: $y = -0.190x + 49.349$. Note that, for the purposes of this figure, all analyses and corresponding equations refer to simple regressions. For statistical tests, see the main text.

Literature Cited

- Alatalo, R. V., L. Gustafsson, and A. Lundberg. 1990. Phenotypic selection on heritable size traits - environmental variance and genetic response. *American Naturalist* 135:464-471.
- Arnold, J. M., J. J. Hatch, and I. C. T. Nisbet. 2004. Seasonal declines in reproductive success of the common tern *Sterno hirundo*: timing or parental quality? *Journal of Avian Biology* 35:33-45.
- Badyaev, A. V., and C. K. Ghalambor. 2001. Evolution of life histories along elevational gradients: trade-off between parental care and fecundity. *Ecology* 82:2948-2960.
- Beldal, E. J., E. Barba, J. A. Gil-delgado, D. J. Iglesias, G. M. Lopez, and J. S. Monros. 1998. Laying date and clutch size of great tits (*Parus major*) in the Mediterranean region: a comparison of four habitat types. *Journal für Ornithologie* 139:269-276.
- Burt, A. 1995. Perspective - the evolution of fitness. *Evolution* 49:1-8.
- Charmantier, A., C. Perrins, R. H. McCleery, and B. C. Sheldon. 2006. Age-dependent genetic variance in a life-history trait in the mute swan. *Proceedings of the Royal Society B-Biological Sciences* 273:225-232.
- Cooke, F., P. D. Taylor, C. M. Francis, and R. F. Rockwell. 1990. Directional selection and clutch size in birds. *American Naturalist* 136:261-267.
- Crick, H. Q. P. 2004. The impact of climate change on birds. *Ibis* 146:48-56.
- Daan, S., C. Dijkstra, and J. M. Tinbergen. 1990. Family-planning in the kestrel (*Falco tinnunculus*) - the ultimate control of covariation of laying date and clutch size. *Behaviour* 114:83-116.
- Drent, R. H., and S. Daan. 1980. The prudent parent - energetic adjustments in avian breeding. *Ardea* 68:225-252.
- Fargallo, J. A. 2004. Latitudinal trends of reproductive traits in the blue tit *Parus caeruleus*. *Ardeola* 51:177-190.
- Garant, D., L. E. B. Kruuk, R. H. McCleery, and B. C. Sheldon. 2004. Evolution in a changing environment: a case study with great tit fledging mass. *American Naturalist* 164:E115-E129.
- Gross, S. J., and T. D. Price. 2000. Determinants of the northern and southern range limits of a warbler. *Journal of Biogeography* 27:869-878.

- Jarvinen, A. 1989. Clutch-size variation in the pied flycatcher *Ficedula hypoleuca*. *Ibis* 131:572-577.
- Katti, M., and T. D. Price. 2003. Latitudinal trends in body size among over-wintering leaf warblers (genus *Phylloscopus*). *Ecography* 26:69-79.
- Krementz, D. G., and P. Handford. 1984. Does avian clutch size increase with altitude? *Oikos* 43:256-259.
- Kruuk, L. E. B., J. Merilä, and B. C. Sheldon. 2001. Phenotypic selection on a heritable size trait revisited. *American Naturalist* 158:557-571.
- Lack, D. L. 1947. Darwin's Finches. Cambridge University Press, Cambridge.
- Magrath, R. D. 1991. Nestling weight and juvenile survival in the blackbird, *Turdus merula*. *Journal of Animal Ecology* 60:335-351.
- Marchetti, K. 2000. Egg rejection in a passerine bird: size does matter. *Animal Behaviour* 59:877-883.
- Meijer, T., S. Daan, and M. Hall. 1990. Family-planning in the kestrel (*Falco tinnunculus*) - the proximate control of covariation of laying date and clutch size. *Behaviour* 114:117-136.
- Merilä, J., L. E. B. Kruuk, and B. C. Sheldon. 2001a. Cryptic evolution in a wild bird population. *Nature* 412:76-79.
- Merilä, J., B. C. Sheldon, and L. E. B. Kruuk. 2001b. Explaining stasis: microevolutionary studies in natural populations. *Genetica* 112:199-222.
- Morrissey, C. A. 2004. Effect of altitudinal migration within a watershed on the reproductive success of American dippers. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* 82:800-807.
- Nogales, M. 1995. Breeding strategies of ravens *Corvus corax* in an oceanic island ecosystem (El-Hierro, Canary-Islands). *Journal für Ornithologie* 136:65-71.
- Perrins, C. M. 1965. Population fluctuations and clutch-size in the great tit, *Parus major*. *Journal of Animal Ecology* 34:601-647.
- Perrins, C. M. 1970. Timing of birds breeding seasons. *Ibis* 112:242-255.
- Perrins, C. M., and R. H. McCleery. 1989. Laying dates and clutch size in the great tit. *Wilson Bulletin* 101:236-253.

- Price, T. 1991. Environmental and genotype-by-environment influences on chick size in the yellow-browed leaf warbler *Phylloscopus inornatus*. *Oecologia* 86:535-541.
- Price, T., and N. Jamdar. 1991a. Breeding biology of the yellowbrowed leaf warbler *Phylloscopus inornatus* in Kashmir. *Journal of the Bombay Natural History Society* 88:1-16.
- Price, T., and N. Jamdar. 1991b. Breeding of eight sympatric species of *Phylloscopus* warblers in Kashmir. *Journal of the Bombay Natural History Society* 88:242-255.
- Price, T., M. Kirkpatrick, and S. J. Arnold. 1988. Directional selection and the evolution of breeding date in birds. *Science* 240:798-799.
- Ringsby, T. H., B. E. Saether, and E. J. Solberg. 1998. Factors affecting juvenile survival in house sparrow *Passer domesticus*. *Journal of Avian Biology* 29:241-247.
- Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society of London Series B-Biological Sciences* 263:1415-1421.
- Rowe, L., D. Ludwig, and D. Schluter. 1994. Time, condition, and the seasonal decline of avian clutch size. *American Naturalist* 143:698-772.
- Sanz, J. J. 1997. Geographic variation in breeding parameters of the pied flycatcher *Ficedula hypoleuca*. *Ibis* 139:107-114.
- Sanz, J. J. 1998. Effects of geographic location and habitat on breeding parameters of great tits. *Auk* 115:1034-1051.
- Sheldon, B. C., L. E. B. Kruuk, and J. Merilä. 2003. Natural selection and inheritance of breeding time and clutch size in the collared flycatcher. *Evolution* 57:406-420.
- Slagsvold, T. 1976. Annual and Geographical Variation in Time of Breeding of Great Tit *Parus-Major* and Pied Flycatcher *Ficedula-Hypoleuca* in Relation to Environmental Phenology and Spring Temperature. *Ornis Scandinavica* 7:127-145.
- Svensson, L. 1992. Identification guide to European passerines, Stockholm.
- Tinbergen, J. M., and M. C. Boerlijst. 1990. Nestling weight and survival in individual great tits (*Parus major*). *Journal of Animal Ecology* 59:1113-1127.
- Tinbergen, J. M., and S. Daan. 1990. Family-planning in the great tit (*Parus major*) - optimal clutch size as integration of parent and offspring fitness. *Behaviour* 114:161-190.

- Van Noordwijk, A. J., R. H. McCleery, and C. M. Perrins. 1995. Selection for the timing of great tit breeding in relation to caterpillar growth and temperature. *Journal of Animal Ecology* 64:451-458.
- Verhulst, S., J. H. Vanbalen, and J. M. Tinbergen. 1995. Seasonal decline in reproductive success of the great tit - variation in time or quality. *Ecology* 76:2392-2403.
- Wardrop, S. L., and R. C. Ydenberg. 2003. Date and parental quality effects in the seasonal decline in reproductive performance of the tree swallow *Tachycineta bicolor*: interpreting results in light of potential experimental bias. *Ibis* 145:439-447.
- Williams, T. D. 1994. Intraspecific variation in egg size and egg composition in birds - effects on offspring fitness. *Biological Reviews of the Cambridge Philosophical Society* 69:35-59.

CHAPTER II

High incidence of extra-pair paternity in a monochromatic, socially monogamous passerine bird *Phylloscopus humei*, based on newly identified microsatellite markers

Abstract

We assessed paternity in Hume's warbler, *Phylloscopus humei*, a sexually monochromatic, socially monogamous passerine, using six new, species-specific microsatellite DNA markers. We analyzed a total of 308 *P. humei* nestlings from two populations—Manali, India (2001, 2003, 2004) and Naran, Pakistan (1999). Overall, the proportion of extra-pair young found in broods was similar in each population: Manali: 35% of nestlings, found in 54% of all broods; Naran: 32% of nestlings from 64% of broods. This percentage of extra-pair young is considerably higher than one might expect for a monochromatic, socially monogamous species. It is, however, similar to levels reported in other studies of *Phylloscopus* warblers, which are also monomorphic, and mostly socially monogamous. We find no evidence that females seek extra-pair fertilizations for the purposes of indirect genetic benefits, increased offspring genetic diversity, or for fertility assurance. Instead, the high level of extra-pair paternity may be a consequence of high breeding density and breeding synchrony combined with high levels of male mortality from one year to the next. These factors together may lead males to aggressively seek copulations outside of the pair bond.

Introduction

In birds, extra-pair paternity, or production of offspring as a result of mating with individuals outside the social bond, is common (Møller & Ninni 1998; Griffith *et al.* 2002). Griffith *et al.* (2002) report that in socially monogamous species of birds, on average extra-pair young comprise 11% of offspring and are present in 19% of broods. Despite extra-pair paternity being both common and widespread, the levels of its occurrence vary considerably across species. Among socially monogamous birds for example, the proportion of extra-pair young ranges from 0% (Griffith *et al.* 2002 and references therein) up to 55% of offspring and 86% of broods in the reed bunting *Emberiza schoeniclus* (Dixon *et al.* 1994). There is currently much interest in assessing the causes of this variation.

Benefits to males in achieving extra-pair fertilization are apparent—males increase their fitness as the number of females they mate with increases (Trivers 1972). One class of explanations for the variation in extra-pair fertilization rate comes from considering potential benefits to females of participating in extra-pair copulations, in terms of survival of their offspring (Petrie & Kempenaers 1998; Griffith *et al.* 2002; Westneat & Stewart 2003; Griffith 2006 *in press*). This includes assurance of fertility (Wetton & Parkin 1991), indirect benefits (i.e., the production of high quality offspring), and genetic compatibility of genes from the father (Tregenza & Wedell 2000; Mays & Hill 2004). A related early observation was that the proportion of extra-pair young in a species is related to plumage brightness and dimorphism--sexually dimorphic species and those with bright plumage have higher levels than do dull monochromatic species (Møller

& Birkhead 1994; Owens & Hartley 1998). Male showiness and hence sexual dichromatism may have explicitly evolved to advertise male genetic quality to attract females for extra-pair copulations (Hamilton 1990; Møller & Birkhead 1994). In support of this possibility, population studies have shown that females may engage in extra-pair copulations with males that are more attractive than their social mate (e.g., have more complex songs [Hasselquist *et al.* 1996; Kempenaers *et al.* 1997], have notes in the song that are difficult to sing [Forstmeier *et al.* 2002], have larger plumage patches [Sheldon *et al.* 1997], or have brighter plumage [e.g., Yezerinac & Weatherhead 1997; Greene *et al.* 2000; reviewed by Griffith *et al.* 2002]). In blue tits, *Parus caeruleus*, and red-winged blackbirds, *Agelaius phoeniceus*, males who were successful cuckolders were older than those who were not (Delhey *et al.* 2003; Weatherhead & Boag 1995). Finally, attractive males maintain a higher proportion of paternity in their nests than less attractive males (Møller & Ninni 1998).

A second class of explanations is that extra-pair copulation behavior results from male harassment and solicitations (Arnquist & Kirkpatrick 2005). This fits some of the clearer correlates of extra-pair paternity rate, including population density, breeding synchronization and high male mortality. High male mortality places an additional premium on males to seek out extra-pair partners in what maybe their final breeding season, and also means that cuckolded males are less likely to withhold paternal care (Mauck *et al.* 1999; Wink & Dyrce 1999; Griffith *et al.* 2002). Some support for these explanations comes from an inability to generally detect differences in fitness between cuckolded and non-cuckolded offspring in a nest (Arnquist & Kirkpatrick 2005) and a general weakening of the correlation between sexual dimorphism and extra-pair

copulation rate as more studies accumulate. For example, in studies with 200 or more young of monochromatic socially monogamous species, the average fraction of broods with extra-pair young was 19%, with 8% of young being extra-pair ($N = 7$ species, Griffith *et al.* 2002 and references therein), which does not differ much from levels of all socially monogamous birds (i.e., 11% of young and 19% of broods).

Within species, levels of extra-pair fertilization may vary across populations and years (reviewed in Petrie & Kempenaers 1998; Griffith *et al.* 2002). A number of ecological factors such as breeding density and synchrony may influence these intraspecific differences, though their effect is unclear, and support for them is equivocal (Griffith *et al.* 2002). Determining which factors are relevant to a particular species may help elucidate the motivations for extra-pair fertilization. Given these differences, single species studies of extra-pair fertilization are most insightful across years and populations.

In this paper, we study the prevalence of extra-pair fertilizations and their potential adaptive correlates in, Hume's warbler, *Phylloscopus humei*, using newly isolated microsatellite DNA loci. The species is sexually monomorphic (Marchetti 1998), socially monogamous, and both males and females contribute to raising the young -- all factors that suggest extra-pair paternity should be low (Griffith *et al.* 2002). However, the species breeds at high density and with high synchrony (Price & Jamdar 1991a) and likely has high adult mortality. These factors may lead to conditions where females are regularly receiving copulation solicitations from females, and hence favor a high rate of extra-pair copulation (Griffith *et al.* 2002). In support of this argument, we show here that extra-pair copulations are high. In addition, there are no obvious adaptive correlates in terms of survival of young. This result favors the second class of

explanations in which the main factor leading to high levels of extra-pair copulation is male solicitation and/or harassment.

Materials and Methods

Species description

Phylloscopus humei is a small (6g), ground nesting passerine that breeds abundantly in the Western Himalaya (Baker 1997). It is socially monogamous, although 3 instances of social bigamy have now been recorded (Price & Jamdar 1991a, and see below). Males arrive on the breeding grounds and compete amongst themselves for territories prior to the arrival of females. Upon arrival, females choose mates, apparently based on a suite of characters rather than on any single male trait (Marchetti 1998). Only females build nests and incubate eggs, but both parents provide care to the offspring once hatched.

P. humei is dull colored, except for pale wing-bars (Marchetti 1993). Sexual size dimorphism is limited. Based on adults measured for this study, on average, males' tarsi are 3% longer than females' ($t_{199} < -6.40$; $P = 0.0001$), and their wings are 8% longer ($t_{134} = -14.56$; $P < 0.0001$). Similar patterns were found in a population of *P. humei* in Kashmir (Price & Jamdar 1991a). The wing-bar is a result of light-colored feather tips of the six greater covert feathers. It is present in both males and females, but it is slightly dimorphic, averaging 14% larger in adult males than adult females in our main study population in Manali ($t_{200} = -2.98$; $P = 0.0033$). Manipulation experiments of male

wing-bar size in the breeding season resulted in corresponding changes to their territory area, indicating that the trait is subject to intra-sexual selection (Marchetti 1993). The wing-bar is displayed in many other interactions (e.g., in the maintenance of winter territories--males and females hold individual territories on their wintering grounds in north and central India; Gross & Price 2000), but its function in females remains to be tested. The wing-bar is fully developed and unfolded in nestlings (at age 10-11 days), so this trait can be studied on both adults and nestlings.

Fieldwork

We sampled broods from Naran, Pakistan ($34^{\circ}75'N$, $73^{\circ}50'E$); $N = 11$ broods (containing 38 nestlings) collected in 1999 and Manali, India ($32^{\circ}14'N$, $77^{\circ}7'E$); $N = 84$ full broods (containing 274 nestlings) in 2001, 2003, 2004 (Table 1). Description of the study area at Manali is given in Price *et al.* (2003). Naran has similar habitat to Manali, but at higher elevation, willow *Salix* rather than rhododendron is the dominant vegetation (Price, *pers. obs.*). In 2003 at Manali, the location and elevation of all nests found was recorded via GPS for calculations of nest distances. In 2004 at Manali, we recorded only the coordinates and elevation of successful nests, so distances between all discovered nests cannot be calculated. Breeding density was not explicitly measured for either population, but the two are thought to be similar to each other (>1 pair/ha, though patchy in distribution).

At Manali, some males were caught on their territories using song playback before breeding began. These males were color banded so that they could be visually confirmed to be the attending males of their nests. In the majority of cases, though, both parents were caught in mist nets at the nest. This means that individuals whose nests failed were not sampled in most cases. At Manali, but not Naran, nestlings (at age 10-11 days) and adults were weighed, and tarsus length measured (the length of the tarsus to the last complete scale (Svensson, 1992). Tarsus length on nestlings has almost reached adult size by this age. On adults, we also measured wing-length (flattened wing chord length). The sex of adult birds is easily told based on presence of a brood patch (only the female incubates). The sex of nestlings was determined using a DNA-based test (see below).

Wing-bar collection and measurement

To estimate wing-bar size, we plucked the fourth greater covert feather from each wing for all birds in the Manali population. Feathers were mounted individually on glass slides and measured in random order in the laboratory under a dissecting microscope. The light-colored portion of the feather was measured down the length of the feather shaft. We used the average of the two feathers (one from each side) as an estimate of the size of an individual's wing-bar.

Blood collection and processing

Blood for genetic analysis was collected by brachial vein puncture. For each bird sampled, a drop of blood was drawn into a capillary tube and then emptied into tubes containing Queen's lysis buffer (Seutin *et al.* 1991) for storage. In rare cases where sufficient blood was not available to draw into a capillary tube, blood was blotted onto filter paper, which was dried by placing the uncapped tube in a container of dessicant. The paper was then placed in dry / empty microcentrifuge tubes for storage. We collected breast muscle from a few nestlings that were found dead in the nest. We also collected any unhatched eggs, stored their contents in lysis buffer, and attempted to extract DNA from embryonic tissue if chick development had begun.

We extracted DNA from both blood and tissue using DNeasy tissue kits (Qiagen). We followed the kit protocol, using enough blood to turn the lysis buffer (provided in the kit) to a straw color. If only dried blood was available, we cut out the portion of filter paper containing the dried blood and dropped into the lysis buffer. Extracted samples were incubated with RNase A overnight at 55°C.

Juveniles were sexed using a DNA test based on sex-specific differences in the sizes of CHD introns. In many birds, the size of an intron of the CHD gene differs on the W and Z chromosomes, and amplifying the portion of those genes using the primers CHD-P2 and CHD-P8 produces products of different size (Griffiths *et al.* 1998). Reactions were 10µl in volume (1x PCR buffer B (Fisher), 1 µg BSA (New England Biolabs), 2.5 mM MgCl₂ (Fisher), 200 µM dNTPs (Promega), 0.5U *Taq* DNA polymerase (Fisher), 100 µM each primer). The thermal profile of the reactions was as

follows: 5 min at 94°C; 35 cycles of 20 sec at 94°C, 20 sec annealing, 45 sec at 72°C extension; 5 min at 72°C. Products were then run out on a 3% agarose gel and amplified products sized by reference to a size standard (100 bp ladder; Invitrogen). Using a behaviorally sexed adult male and female as positive controls, we determined that in *P. humei*, amplification of this region in females resulted in two bands approximately 340 and 400 base pairs in length; amplification of male DNA produced only the c. 340 base pair band.

Microsatellite isolation, primer design and genotyping

To isolate species-specific microsatellite loci, DNA from two females (i.e., the heterogametic sex) was sent to Savannah River Ecology Labs where potential loci were isolated, as described by Glenn & Schable (2005). Specifically, the DNA was enriched for repeat sequences, and enriched fragments were cloned into plasmids and sequenced. Sequences were then checked visually to find clean repeats for which primers could be designed based on flanking sequences. We designed candidate primers using the web interface of Primer3 (Rozen & Skaletsky 2000). The program Oligo (Molecular Biology Insights, Cascade, CO) was also used to check for duplex formation, hairpins, and false priming sites in the returned possibilities. Primer sequences are given in Table 2.

Using the DNA from a randomly selected adult as template, amplification of fragments containing repeats was run with the selected primers over a gradient of annealing temperatures between 50° – 60°C. Using this approach, we determined that an annealing temperature of 56°C was optimal for all primer sets. The reaction mixture and

thermal profile, excepting annealing temperature, were the same as in sexing reactions. We then ordered 5'-fluorescently labeled forward primers for primer sets that yielded a clean product within the gradient of annealing temperatures. Dyes used to label primers were HEX, 6-FAM (Sigma-Genosys) and NED (Applied Biosystems).

We first genotyped approximately 20 adults at each locus, as described above. Based on their genotypes, we then estimated observed (H_{obs}) and expected (H_{exp}) heterozygosity using the web interface of Genepop (Laboratoire de Genetique et Environment, Montpellier, France; wbimed.curtin.edu.au/genepop). Null allele frequency was estimated as $r = (H_{exp} - H_{obs}) / (1 + H_{exp})$, where r is the proportion of null alleles (Brookfield 1996). The reverse primer of one locus (Phu1-9E) was redesigned after obtaining a null allele frequency of 16%. Six polymorphic loci with low null allele frequency (i.e., < 7%) were used for genotyping and paternity analysis (Table 2).

The complete dataset was obtained using a PCR multiplexing kit (Qiagen) to simultaneously amplify all loci except for Phu1-9E and Phu4-86, following directions in the kit manual. These loci were amplified separately. Microsatellite genotyping was conducted using ABI 3100 and 3700 sequencers and GeneScan and GeneMapper software, using a ROX 350 or 500 base pair standard (Applied Biosystems). All individuals sampled in the Naran population were successfully genotyped. Presumably because of low quality DNA, we were unable to obtain data from enough loci to estimate paternity of 6 of 14 young in the Manali population that were either found dead (Table 1) or as embryos in unhatched eggs.

Paternity analysis

Allele sizes of all individuals were entered into CERVUS (Marshall *et al.* 1998; Slate *et al.* 2000) after rounding them to the nearest integer. Although females were caught when on the nest or feeding young, we were concerned that errors (e.g., mis-labeling) could result in mis-assignment of some parents, which would inevitably inflate our estimate of extra-pair fertilizations. To estimate the error rate, neither parent was labeled as known, and all sampled adults in each population/year were considered candidate parents. Gene frequency data were generated for the analyses using only the adults of each population (Table 3). Candidate parents were prioritized by highest number of matches and lowest number of mismatches with the offspring genotype, and most-closely matching females and males were compared by eye with the offspring genotype to determine which combination of male and female were the likely genetic parents. We used this approach rather than using the likelihood score that CERVUS calculates because the program often assigned most likely mothers as females whose genotypes less closely matched those of the young than females who were actually caught at the nest. The result of this analysis is that we were able to match all females to young in the nest they attended. Thus, we feel confident that errors were small, and not likely to inflate our estimates of extra-pair paternity.

After assigning mothers, the most-closely matching fathers (as output by CERVUS, using the criteria described above) were compared to the attending male at the nest. If this most likely father was not the attending male, we visually checked the offspring and the mother's genotype to confirm this male as the father. For extra-pair

young whose genetic fathers were not identified in 2003 and 2004, paternity analysis was repeated with mothers assigned as described above and with candidate fathers including males who had been sampled as nestlings in previous years, in case there were year old returning males that we did not capture.

Correlates of extra-pair fertilizations

In the Manali population, we assessed possible relationships between extra-pair paternity and wing length (in adults), wing-bar size and tarsus length (in all birds), and genetic variability of nestlings. Tarsus length of young has been correlated with their survival probability (e.g., Alatalo *et al.* 1990; Ringsby *et al.* 1998), and might thus be used as a general measure of condition. To estimate genetic diversity of an individual, we calculated observed heterozygosity and mean d^2 (Coluson *et al.* 1998) for all young genotyped. Heterozygosity was calculated as the proportion of loci heterozygous. For an individual locus, d^2 is the squared difference in repeat unit length between alleles (Coulson *et al.* 1998).

For 2003, nest locations that were recorded in decimal degrees were converted to coordinates in Universal Transverse Mercator system using ArcGIS v9 (ESRI, Redlands, CA). Pairwise distances between nests were computed using the Point Distance command.

We used JMP IN (SAS Institute, Cary, NC) to analyze morphological, genetic, and nest data in the Manali population. We tested 1) characteristics of fathers attending nests with and without extra-pair young, 2) characteristics of extra-pair young vs. within-

social pair young and 3) characteristics of males known to obtain extra-pair copulations, although sample sizes are small for this last analysis. For all comparisons of cuckolded with non-cuckolded males, year was included as a factor in the analyses. For comparisons of within-pair with extra-pair young, paired tests were conducted in which the mean trait value among within-pair nestlings and among extra-pair nestlings were calculated and compared for each nest.

Even weak adaptive correlates may be potentially important in driving extra-pair copulation behavior if such behavior comes with few costs. This means that if we fail to reject the null hypothesis, statistical power becomes an important issue. We calculated the power of all tests as the smallest difference between groups that would be required to detect a difference (at $\alpha = 0.05$), given the variability in the sample, and the same sample sizes. These analyses were also conducted in JMP IN.

Results

Mating system

The mating system is confirmed to be social monogamy. At Manali, of the 88 nests from which both parents and offspring were sampled, we trapped a single male and female at 83 of them. In the other 5 cases, only a single parent was observed caring for offspring (4 females and 1 male); young from these nests were excluded from our analysis. Only one clear instance of social bigamy was observed. In this case, we caught a male with playback on one territory but observed him feeding nestlings on a different

territory later in the season. At the time the nestlings were sampled, the mother alone tended the nest in the original territory. Also excluded from our main analysis were unhatched embryos and juveniles found dead, in case of any increased risk of mortality of either extra- or within-pair young.

Extra-pair fertilizations

The overall percentage of extra-pair young in Manali was 35% with 54% of broods containing ≥ 1 extra-pair young: in Naran these values were 31% and 64%, respectively (Table 4). Within families with extra-pair young, 66% of offspring in Manali and 55% in Naran tended to be extra pair. The level of extra-pair fertilization varies little across years or across populations. Of the 8 dead juveniles and embryos that we were able to genotype fully or in part (who were not included in our calculations of percentages of extra-pair young), 5 matched their social parents, and 3 did not.

Correlates of extra-pair fertilizations

We first compared nests with or without extra-pair young. Broods containing extra-pair young did not differ in banding date ($t_{79} = 0.067$; $P = 0.95$), elevation ($t_{78} = -0.71$; $P = 0.48$), or brood size ($\chi^2_2 = 2.92$; $P = 0.23$). Heterozygosity (ranked from 0 to 6 according to the number of loci that were heterozygous), did not differ between extra-pair and within-pair young (controlling for year, $\chi^2_6 = 8.49$; $P = 0.20$), and neither did mean d^2 ($t_{307} = -0.082$; $P = 0.93$).

Male traits

At Manali, we were able to identify only 10 males who had fathered extra-pair young, accounting for 25 of 85 (29%) extra-pair offspring. Three of these nest pairs for which the extra-pair father was identified were from 2003, and we were thus able to determine distances from them to every other nest found in that year. In each of these cases, the cuckolding male's social nest was not the nearest neighboring nest to that which contained his extra-pair young.

In three cases, the extra-pair father was not captured in the year he sired extra-pair young. Because such traits as wing length, weight, and wing-bar size differ from year to year, these three males could not be compared in this analysis, leaving just 7 males to test. Treating the cuckolded male and the cuckolder of a given nest as a matched pair, we found no difference among males of each group (wing-bar, $t_6 = 1.52$; $P = 0.18$; tarsus, $t_6 = -1.16$; $P = 0.29$; weight, $t_4 = -0.62$; $P = 0.57$; for wing, $t_6 = 0.56$; $P = 0.59$). However, the power of this analysis is low.

Larger sample sizes are available to compare the characters of social males who did and did not lose paternity in their nests, with power to detect differences of 1% - 10%, depending on the character examined (Table 5). However, no differences are apparent in the morphological measurements between males who lost and did not lose paternity in their nests (Table 5). Finally, we have power to detect differences of a few percent in comparisons of extra-pair young with their nestmates, who were fathered by the attending male. However, differences are small and not significant (Table 6).

Finally, we found a similar sex ratio between extra-pair (53% female) and within-pair young (55% female; controlling for year $\chi^2_2 = 1.99$; $P = 0.37$).

Discussion

Griffith *et al.* (2002) recommend sampling at least 200 offspring in a study of parental assessment in birds. We sampled > 300 offspring. Our results indicate one-third of all nestlings are sired by males who are not the social father, and this pattern is similar across multiple years and different locations. Other species of *Phylloscopus* warblers show many of the same patterns observed in *P. humei*. For example, all are monomorphic, and they are often some of the commonest birds in the communities where they occur. In two species, the willow warbler *P. trochilus* and the dusky warbler *P. fuscatus*, extra-pair paternity rates are similarly high (Table 7). However, in a study of a different population of *P. trochilus*, as well as a population of *P. sibilatrix*, Gyllenstein *et al.* (1990) found very low levels of extra-pair paternity (Table 7). The low extra-pair paternity rates in the *P. trochilus* population has been attributed to low breeding density (Bjørnstad & Lifjeld 1997; Fridolfsson *et al.* 1997) and the unusual denseness of vegetation creating low visibility in that habitat (Fridolfsson *et al.* 1997), but the low rate in *P. sibilatrix* remains unexplained. All of these warblers are sexually monochromatic, and all but *P. sibilatrix* (in which in one study, 23% of males were found to be polygynous; Temrin & Jakobsson 1998) are socially monogamous.

In the introduction, we suggested two classes of explanations for high rates of extra-pair copulation. First, females may benefit in terms of increased survival of offspring (Griffith *et al.* 2002, Griffith 2006 *in press*). Second, extra-pair copulations are a result of male harassment and solicitation (Arnqvist & Kirkpatrick 2005). We find more support for the second class of explanations. Despite reasonable power, we could not detect differences in genetic variability between extra-pair and within-pair young; nor can we detect differences in the traits of cuckolded and non-cuckolded males. This is consistent with the recent analysis of Arnqvist & Kirkpatrick (2005) of 6 bird species (based on data compiled from 11 published studies), which found no significant positive indirect selection in females for extra-pair copulation behavior, suggesting that, in general, there is no real benefit to offspring in being extra-pair.

We were unable to capture all *P. humei* adults breeding at our study site. Most adults that we sampled were caught while tending nests, but many nests were abandoned or predated prior to our sampling and not all nests were discovered. In 2003, for example, of 64 nests discovered, 33 were sampled (this includes families for which only a single parent was caught), 10 were abandoned, and 21 were predated. More than 30% of nests fell to predation (many were likely to have been predated before we discovered them), and males associated with these nests were not sampled. In addition, it appears that females may obtain extra-pair copulations from distant males, as supported by the three cases in which extra-pair fathers were identified and nest distances were known. Together, this presumably explains why only 29% of extra-pair offspring could be assigned fathers in our sample. Because so few matches were made, we were unable to characterize males who successfully obtain extra-pair fertilization. Several studies have

found differences between males that achieve extra-pair paternity and those that do not (e.g., Hasselquist *et al.* 1996; Kempenaers *et al.* 1997; Yezerinac & Weatherhead 1997). However this does not necessarily mean that females are actively seeking out certain males to mate with. Instead they may be visited more often, be more stimulated, or more harassed, by such males.

Our failure to detect differences between within- and extra-pair young and cuckolded and non-cuckolded fathers may be because we did not measure the correct traits. For example, in great reed warblers, *Acrocephalus arundinaceus*, females who produced extra-pair young obtained extra-pair fertilization from males with larger song repertoires than their social mates, and post-fledging survival of offspring was correlated to paternal song repertoire size (Hasselquist *et al.* 1996). In bluethroats, *Luscinia svecica*, extra-pair offspring were found to have higher immune T-cell-mediated immunity (Johnsen *et al.* 2000). Characters such as these were not included in our study. However, a male's quality is likely to be reflected in many correlated traits, including some, such as wing-bar size, that we did measure. Marchetti (1998) found that females based their choice of social mate on a suite of characters.

We suggest that in this population, high adult mortality, high breeding synchrony, and high density probably are the main factors contributing to high extra-pair paternity in *P. humei*. We consider each in turn. High male mortality is thought to generally correlate with extra-pair copulation rates because females are less likely to be subject to retaliation for extra-pair copulation by their social mate—as the likelihood of a male's survival increases, he suffers less fitness consequence to withholding care to a brood which may include offspring that are his (Mauck *et al.* 1999). In addition, we suggest

that if males have low survival probability, they may invest more in seeking out extra-pair females in what may be their last breeding attempt. We do not have direct estimates of survival, but the species is very small (6g), and small size is generally correlated with low survival in birds (Sæther 1987; Sæther 1989). Marchetti (1998) also reports that only ~ 30% of males return from one year to the next in Overa, Kashmir. Because of dispersal and a failure to recapture all birds, this underestimates adult survival but is consistent with relatively high adult mortality.

Breeding synchrony, which is the proportion of simultaneously fertile females in a population, may affect extra-pair paternity rates in *P. humei*. Some studies have shown a positive association between breeding synchrony and rates of extra-pair paternity (reviewed in Griffith *et al.* 2002). This may result from females being able to simultaneously assess potential extra-pair mates in synchronously breeding populations (Stutchbury & Morton 1995), possibly coincident with no large tradeoff to males between mate guarding and pursuing extra-pair fertilizations (Stutchbury *et al.* 1997). There are no experimental data to support a causal relationship (Griffith *et al.* 2002), however. The fertile period of a female is defined as the time from pair formation until the day that the penultimate egg is laid (Stutchbury & Morton 1995). More than 98% of *P. humei* nests we found had juveniles hatching within a two-week period. Given the length of time (sometimes as long as two weeks, *pers. obs.*) between the start of nest building (which occurs after pair formation) and when the first egg is laid, it appears that the breeding of *P. humei* is highly synchronous.

Breeding density might also help explain the high level of extra-pair paternity detected in this study. *P. humei* is one of the seven most common of all bird species at Manali (Price *et al.* 2003) and is commonest species at another study site in Overa, Kashmir (Price & Jamdar 1991b). It seems likely that this high density is related to the high level of extra-pair fertilization, with males easily able to locate females. One explanation for the low level of extra-pair fertilizations in *P. trochilus* found by Gyllensten *et al.* (1990) is the low visibility in their habitat and low breeding density (0.58 pairs/ha; Fridolfsson *et al.* 1997).

To summarize, in this study, we have identified a high level of extra-pair paternity in a monochromatic, socially monogamous passerine. This high rate is best explained by high adult mortality, high population density, and high breeding synchrony in this species, and evidence that females are seeking out males that provide particular benefits in terms of enhanced offspring fitness has less support.

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The text of Chapter II, in full, is a reprint of the material as it has been submitted to the journal *Molecular Ecology*. I was the primary author of this chapter. The co-authors listed in this publication directed and supervised the research in this chapter.

Bontrager, A. L., H.L. Gibbs, and T. D. Price. (submitted). High incidence of extra-pair paternity in a monochromatic, socially monogamous passerine bird *Phylloscopus humei*, based on newly identified microsatellite markers. *Molecular Ecology*.

Table II.1. Numbers of adult males (M), adult females (F), and nestlings (n) sampled in each year. Full families are those in which all young and both parents were sampled. Partial families are those in which a parent or one or more nestlings was missing from the sample. "Other indicates samples that do not fit the other two descriptions; this includes parents whose nests were predated before the young could be sampled, nestlings whose parents were not sampled, and nestlings found dead. In 2003, one male was caught tending two separate nests. This is reflected in the discrepancy in number of males and females in full families that year. Recaptured individuals are given in parentheses.

Year	Full families			Partial families			Other			Total		
	M	F	n	M	F	n	M	F	n	M	F	n
Manali 2001	29	29	96	0	1	2	4	0	0	33	30	98
Manali 2002	0	0	0	0	0	0	5(2)	3	0	5(2)	3	0
Manali 2003	25(3)	26(3)	105	1	3	10	7(2)	2	6	32(5)	33(3)	121
Manali 2004	19(6)	16(9)	69	2	2	3	1	1	16	22(6)	19(9)	90
Naran 1999	11	11	38	0	0	0	2	0	0	11	11	38

Table II.2. Characteristics of *Phylloscopus humei* microsatellite markers. F indicates the forward, 5' primer; R indicates the reverse, 3' primer. In the case of *Phu* 1-9E, R1 was the original 3' primer that resulted in the high percentage of null alleles. R2 was the subsequent 3' primer designed that was used in the genotyping analysis.

Locus	Primer sequence	Repeat	Product size
<i>Phu</i> 1-9C	F: AACCTGCTTGAAACGCATC		
	R: AAGGAATTGCAAGTCACAAAGG	(GAA) ₂₄	245
<i>Phu</i> 1-9E	F: GATTTCTACATGGCTGTGAGAGG		
	R1: ATAGCTATGCCCTAGCAGACATCC	(TAGG) ₈ (TAGA) ₉	155
	R2: AATGTGATTTGGAGGCTGTATG		193
<i>Phu</i> 1-10E	F: CTCCAATAGTGACAAGTTTCATGTG		
	R: TGTTGTCCAGTGCATGTG	(CAA) ₁₁	219
<i>Phu</i> 2-44	F: GTTGAAAGCAGAAATGCAATGG		
	R: ACGGCCTCATCTTTGAAATC	(GAA) ₁₃	184
<i>Phu</i> 3-12	F: CTGGCATGTAAACTCTGTGACTG		
	R: GTCTGCAAAAGGCTAGAAATGTTG	(CAAA) ₁₀	170
<i>Phu</i> 4-86	F: AAAGCCTAGAGGAGAAACACCAAG		
	R: TCCTGAAAGTGTGACATCTGACC	(AGAT) ₁₂ AGACAGAT(AGAC) ₃ (AGAT) ₂	186

Table II.3. Results of gene frequency analysis as performed by CERVUS on adults of each population. Ex P1 indicates the exclusion probability if neither parent is known, whereas Ex P2 indicates exclusion probability of the second parent, assuming that the first is known. He is expected heterozygosity, and H_o is observed heterozygosity. Null indicates the proportion of alleles expected to be null.

Locus	Phu 4-86		Phu 1-10E		Phu 2-44		Phu 1-9C		Phu 3-12		Phu 1-9E		All	
Population	Manali	Naran	Manali	Naran	Manali	Naran	Manali	Naran	Manali	Naran	Manali	Naran	Manali	Naran
No. alleles	21	11	12	9	16	9	80	9	22	11	35	16		
Ex P1	0.586	0.519	0.192	0.201	0.465	0.413	0.921	0.826	0.715	0.543	0.732	0.707	0.9989	0.9947
Ex P2	0.739	0.686	0.374	0.383	0.639	0.59	0.959	0.905	0.833	0.706	0.845	0.828	0.9999	0.9996
H_e	0.87	0.862	0.567	0.591	0.815	0.803	0.982	0.979	0.92	0.871	0.925	0.936	0.847	0.84
H_o	0.815	0.818	0.525	0.583	0.89	0.792	0.859	0.947	0.94	1	0.939	0.955		
Null	0.0325	0.0122	0.0424	-0.015	-0.0503	-0.0012	0.0655	0.0024	-0.013	-0.0849	-0.009	-0.0241		

Table II.4. Summary of paternity analysis results for both populations of *P. humei*. Proportion of extra-pair young (EPY) is reported with the 95% confidence interval, which was calculated assuming a binomial distribution of extra-pair young.

Year	Location	No. nests	Prop. with EPY	No. nestlings	Prop. EPY
2001	Manali	29	0.59	96	0.37 ± 0.097
2003	Manali	29	0.55	105	0.33 ± 0.090
2004	Manali	25	0.48	69	0.32 ± 0.11
combined	Manali	83	0.54	263	0.35 ± 0.058
1999	Naran	11	0.64	38	0.32 ± 0.15
total	both	94	0.55	308	0.34 ± 0.053

Table II.5. Comparison of traits between males who did (cuckolded) and did not (not cuckolded) lose paternity in their nests. Power analysis indicates the difference in mean detectable with power of 0.95. The actual difference in sample mean is given in parentheses.

Trait	Male	Mean $\pm$ S.E.	Test statistic	P	Power analysis
Wingbar	not cuckolded	1.40 $\pm$ 0.063	$t_{80} = -0.93$	0.36	10.60% (5.0%)
	cuckolded	1.47 $\pm$ 0.053			
Tarsus	not cuckolded	17.88 $\pm$ 0.089	$t_{80} = -0.82$	0.41	1.34% (0.5%)
	cuckolded	17.98 $\pm$ 0.097			
Wing	not cuckolded	58.47 $\pm$ 0.27	$t_{79} = 0.0050$	0.99	2.82% (0.01%)
	cuckolded	58.48 $\pm$ 0.25			
Weight	not cuckolded	5.94 $\pm$ 0.062	$t_{78} = -0.17$	0.87	1.13% (0.16%)
	cuckolded	5.93 $\pm$ 0.068			

Table II.6. Comparison of traits in within- (WPY) and extra-pair (EPY) nestlings. EPY and WPY values were matched pairswithin nestsm using average values of all nestlings within a nest falling into each category. Power analysis indicates thedifference in mean detectable with power of 0.95. The actual sample mean is in parentheses.

Trait	Nestlings	Mean $\pm$ S.E.	Test statistic	P	Power analysis
Wingbar	WPY	2.16 $\pm$ 0.06	$t_{29} = -0.54$	0.59	9.75%
	EPY	2.13 $\pm$ 0.05			(1.4%)
Tarsus	WPY	17.13 $\pm$ 0.15	$t_{29} = 1.01$	0.32	3.19%
	EPY	17.28 $\pm$ 0.11			(0.8%)
Weight	WPY	6.72 $\pm$ 0.14	$t_{29} = 0.74$	0.47	4.24%
	EPY	6.78 $\pm$ 0.14			(0.9%)

Table II.7. Summary of other paternity studies in *Phylloscopus* warblers. EPY indicates the level of extra-pair young, while broods indicates the proportion of broods containing them. EPM correlates indicates any traits that were found to correlate with successful extra-pair paternity.

Species	Broods	EPY	Markers	EPM correlates	Reference
<i>P. sibilatrix</i>	0 (13)	0 (56)	multilocus minisatellite		Gyllenstein <i>et al.</i> 1990
<i>P. trochilus</i>	0 (19)	0 (120)	multilocus minisatellite		Gyllenstein <i>et al.</i> 1990
<i>P. trochilus</i>	50% (20)	33% (109)	multilocus DNA fingerprinting	higher body mass	Bjørnstad and Liffield 1997
<i>P. trochilus</i>	58% (12)	28% (68)	microsatellites		Fridolfsson <i>et al.</i> 1997
<i>P. fuscatus</i>	59% (46)	45% (195)	microsatellites	high sound amplitude	Forstmeier <i>et al.</i> 2002

Literature Cited

- Alatalo RV, Gustafsson L, Lundberg A (1990) Phenotypic selection on heritable size traits - environmental variance and genetic response. *American Naturalist*, **135**, 464-471.
- Arnqvist G, Kirkpatrick M (2005) The evolution of infidelity in socially monogamous passerines: The strength of direct and indirect selection on extrapair copulation behavior in females. *American Naturalist*, **165**, S26-S37.
- Baker K (1997) *Warblers of Europe, Asia and North Africa*, Princeton University Press, Princeton.
- Bjørnstad G and Lifjeld JT (1997) High frequency of extra-pair paternity in a dense and synchronous population of willow warblers *Phylloscopus trochilus*. *Journal of Avian Biology*, **28**, 319-324.
- Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology*, **5**, 453-455.
- Coulson TN, Pemberton JM, Albon SD, *et al.* (1998) Microsatellites reveal heterosis in red deer. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 489-495.
- Delhey K, Johnsen A, Peters A, Andersson S, Kempenaers B (2003) Paternity analysis reveals opposing selection pressures on crown coloration in the blue tit (*Parus caeruleus*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 2057-2063.
- Dixon A, Ross D, Omalley SLC, Burke T (1994) Paternal investment inversely related to degree of extra-pair paternity in the reed bunting. *Nature*, **371**, 698-700.
- Forstmeier W, Kempenaers B, Meyer A, Leisler B (2002) A novel song parameter correlates with extra-pair paternity and reflects male longevity. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **269**, 1479-1485.
- Fridolfsson AK, Gyllenstein UB, Jakobsson S. (1997) Microsatellite markers for paternity testing in the Willow warbler *Phylloscopus trochilus*: high frequency of extra-pair young in an island population. *Heredity*, **126**, 127-132.
- Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202-222.

- Greene E, Lyon BE, Muehler VR, *et al.* (2000) Disruptive selection for plumage coloration in a passerine bird. *Nature*, **407**, 1000-1003.
- Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, **11**, 2195-2212.
- Griffith SC (2006) The evolution of infidelity in socially monogamous passerines: neglected components of direct and indirect selection and the genetically loaded raffle. *American Naturalist*, in press.
- Griffiths R, Double MC, Orr K, and Dawson RJG (1998) A DNA test to sex most birds. *Molecular Ecology*, **7**, 1071-1075.
- Gross SJ and Price TD (2000) Determinants of the northern and southern range limits of a warbler. *Journal of Biogeography*, **27**, 869-878.
- Gyllenstein UB, Jakobsson S, Temrin H (1990) No evidence for illegitimate young in monogamous and polygynous warblers. *Nature*, **343**, 168-170.
- Hamilton WD (1990) Mate choice near or far. *American Zoologist*, **30**, 341-352.
- Hasselquist D, Bensch S, vonSchantz T (1996) Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature*, **381**, 229-232.
- Johnsen A, Andersen V, Sunding C, Lifjeld JT (2000) Female bluethroats enhance offspring immunocompetence through extra-pair copulations. *Nature*, **406**, 296-299.
- Kempnaers B, Verheyen GR, Dhondt AA (1997) Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behavioral Ecology*, **8**, 481-492.
- Marchetti K (1993) Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature*, **362**, 149-152.
- Marchetti K (1998) The evolution of multiple male traits in the yellow-browed leaf warbler. *Animal Behaviour*, **55**, 361-376.
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639-655.
- Mays HL, Hill GE (2004) Choosing mates: good genes versus genes that are a good fit. *Trends in Ecology & Evolution*, **19**, 554-559.

- Mauck RA, Marschall EA, Parker PG (1999) Adult survival and imperfect assessment of parentage: Effects on male parenting decisions. *American Naturalist*, **154**, 99-109.
- Møller AP, Birkhead TR (1994) The evolution of plumage brightness in birds is related to extrapair paternity. *Evolution*, **48**, 1089-1100.
- Møller AP, Ninni P (1998) Sperm competition and sexual selection: a meta-analysis of paternity studies of birds. *Behavioral Ecology and Sociobiology*, **43**, 345-358.
- Owens IPF, Hartley IR (1998) Sexual dimorphism in birds: why are there so many different forms of dimorphism? *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**, 397-407.
- Petrie M, Kempenaers B (1998) Extra-pair paternity in birds: explaining variation between species and populations. *Trends in Ecology & Evolution*, **13**, 52-58.
- Price T, Jamdar N (1991a) Breeding biology of the yellowbrowed leaf warber *Phylloscopus inornatus* in Kashmir. *Journal of the Bombay Natural History Society*, **88**, 1-16.
- Price T, Jamdar N (1991b) The breeding birds of Overa Wildlife Sanctuary, Kashmir. *Journal of the Bombay Natural History Society*, **87**, 1-15.
- Price T, Zee J, Jamdar K, Jamdar N (2003) Bird species diversity along the Himalaya: a comparison of Himachal Pradesh with Kashmir. *Journal of the Bombay Natural History Society*, **100**, 394-410.
- Ringsby TH, Saether BE, Solberg EJ (1998) Factors affecting juvenile survival in house sparrow *Passer domesticus*. *Journal of Avian Biology*, **29**, 241-247.
- Rozen S, Skaletsky HJ (2000) *Primer3 on the WWW for general users and for biologist programmers*. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. (eds Krawetz S and Misener S), pp. 365-386. Humana Press, Totowa, NJ.
- Sæther BE (1987) The influence of body weight on the covariation between reproductive traits in European birds. *Oikos*, **48**, 79-88.
- Sæther BE (1989) Survival rates in relation to body weight in European birds. *Ornis Scandinavica*, **20**, 13-21.
- Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology-Revue Canadienne de Zoologie*, **69**, 82-90.

- Sheldon BC, Rasanen K, Dias PC (1997) Certainty of paternity and paternal effort in the collared flycatcher. *Behavioral Ecology*, **8**, 421-428.
- Slate J, Marshall T, Pemberton J (2000) A retrospective assessment of the accuracy of the paternity inference program CERVUS. *Molecular Ecology*, **9**, 801-808.
- Stutchbury BJ, Morton ES (1995) The effect of breeding synchrony on extra-pair mating systems in songbirds. *Behaviour*, **132**, 675-690.
- Stutchbury BJM, Piper WH, Neudorf DL, *et al.* (1997) Correlates of extra-pair fertilization success in hooded warblers. *Behavioral Ecology and Sociobiology*, **40**, 119-126.
- Svensson L (1992) *Identification guide to European passerines*, Stockholm.
- Temrin H, Jakobsson S (1998) Female reproductive success and nest predation in polyterritorial wood warblers (*Phylloscopus sibilatrix*). *Behavioral Ecology and Sociobiology*, **23**, 225-231.
- Tregenza T, Wedell N (2000) Genetic compatibility, mate choice and patterns of parentage: Invited review. *Molecular Ecology*, **9**, 1013-1027.
- Trivers RL (1972) *Parental investment and sexual selection*. In: *Sexual Selection and the Descent of Man*, (ed B Campbell), pp. 136-179. Aldine, Chicago.
- Weatherhead PJ, Boag PT (1995) Pair and extra-pair mating success relative to male quality in red-winged blackbirds. *Behavioral Ecology and Sociobiology*, **37**, 81-91.
- Westneat DF, Stewart IRK (2003) Extra-pair paternity in birds: Causes, correlates, and conflict. *Annual Review of Ecology Evolution and Systematics*, **34**, 365-396.
- Wetton JH, Parkin DT (1991) An association between fertility and cuckoldry in the house sparrow, *Passer domesticus*. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **245**, 227-233.
- Wink M, Dyrce A (1999) Mating systems in birds: a review of molecular studies. *Acta Ornithologica*, **34**, 91-109.
- Yezerinac SM, Weatherhead PJ (1997) Extra-pair mating, male plumage coloration and sexual selection in yellow warblers (*Dendroica petechia*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **264**, 527-532.

CHAPTER III

Genetic correlation between the sexes in the color pattern of a sexually monochromatic bird

Abstract

The traditional explanation for the presence of similar ornamental traits in females as males has been a genetic correlation between the sexes, although selection may act to modify expression in the two sexes. Both sexes of the warbler *Phylloscopus humei* possess ornamental wing-bars, and dimorphism is slight (males have wing-bars that are 4.37% larger than females'), which may be associated with a small sex difference in wing length. We calculated the genetic correlation between the sexes of wing-bar size based on father-daughter and mother-son regressions, and obtained estimates of 1.30 and 0.76 respectively; both estimates are significantly different from zero and not significantly different from 1.0, but they are also significantly different from one another. The high correlation suggests that the evolution of the wing-bar in the two sexes is constrained, as found in other studies. The differences between the two regressions imply a possible sex-specific maternal effect. We suggest that the high genetic correlation between the sexes in wing-bar size is sufficient to strongly affect the evolution of the trait in females, but that dimorphism could rather easily change as a result of environmental impacts on development

Introduction

A striking feature of many birds is the degree to which female plumage patterns resemble those of the males of the same species (Amundsen 2000; Amundsen and Pärn 2006). The most commonly considered explanation for this is that, as a result of a high genetic correlation between the sexes, a trait favored as a result of sexual selection on males spreads in females as well. Subsequent sex-specific selection may modify the expression of the trait in females (Fisher 1958, Chapter 6; Lande 1980), but the rate at which this happens depends on both the intensity of selection, and the magnitude of the genetic correlation (Lande 1980).

Sexual dichromatism in birds has evolved frequently (Peterson 1996; Price and Birch 1996; Figuerola and Green 2000). For example, Peterson (1996) identified 158 examples of conspicuous geographical variation in sexual dichromatism (usually one population is dimorphic and another apparently monomorphic). If the genetic correlation between the sexes is high, rapid and frequent evolution of sexual dichromatism is paradoxical. Badyaev (2002) has noted a similar paradox with respect to the evolution of sexual size dimorphism. Several solutions have been suggested. First, the genetic correlation may not be particularly high; for example, a genetic correlation of 0.9 should still allow evolution of dimorphism, given sufficient time. Second, selection may act on sex-specific growth patterns, which may be tightly connected to the relatively small portion of the genetic variance that is sex-limited in adults (Badyaev 2002). Third, genetic correlations between the sexes may occasionally be relaxed, or the genetic variance may increase in one sex but not the other, as a result of stochastic fluctuations of

sex-limited alleles (Reeve and Fairbairn 2001) or occasional sex-limited mutations of major effect (e.g., Price 2002), resulting in rapid evolution over short time intervals. Fourth, Badyaev (2002, 2005a) has suggested that various environmental factors may affect the degree of sexual dimorphism. In particular, sex-specific maternal effects (i.e., influences of the mother on one sex but not the other) may be common (Badyaev 2005a). Such environmental effects could potentially become genetically assimilated over long time intervals (e.g., Badyaev 2005b), again a result of selection on the relatively small part of the genetic variance that is sex limited.

In this paper, we investigate inheritance of a sexually monochromatic trait in Hume's leaf warbler, *Phylloscopus humei*—a pale wing-bar on the wing, which is exhibited during social displays (Marchetti 1993). Monochromatism in this species is surprising because previous experimental work has demonstrated that male wing-bar size is subject to sexual selection by male competition (Marchetti 1993). Manipulation of wing-bar size in males through painting experiments revealed a role of the trait in intrasexual competition (Marchetti 1993). Males with reduced wing-bars either lost territory area or lost territory altogether, ending up, on average, with the smallest territories. Males with enlarged wing-bars gained area and had the largest territories. The function of the wing-bar in females is unknown, although females defend individual territories in the non-breeding season in the winter quarters (Gross and Price 2000).

We find that estimates of the genetic covariances between the sexes for wing-bar size are similar to estimates of the within-sex genetic variances, suggesting a high genetic correlation. This may constrain the evolution of wing-bar size, but we also find that the genetic covariance estimated from father-daughter resemblance is almost twice as high as

that estimated from mother-son resemblance. This large difference suggests the possibility of sex-specific maternal effects, which could rapidly alter sex-limited expression of the trait.

Materials and methods

Species description

Phylloscopus humei is a small (6g), socially monogamous, ground-nesting leaf warbler that breeds abundantly in the western Himalaya (Price and Jamdar 1991a; Rasmussen and Anderton 2005). The species is socially monogamous. Adults are essentially monomorphic in size; males have, on average, longer tarsi and wings than females, though the distribution of these features overlaps considerably in the sexes (Price and Jamdar 1991b; Bontrager et al., submitted).

Males and females are similarly ornamented. In many species, reflectance measurements often indicate small differences between the sexes (Eaton 2005), but we have been unable to detect such differences between the sexes, either in adults or in nestlings (unpublished data). The birds are mostly grayish green with buff colored underparts, and each of their wings has a minor and major light-colored wing-bar. The wing-bars are formed from the lack of eumelanin pigmentation on the tips of the covert feathers (see illustration in Price and Pavelka 1996). The upper, minor wing-bar comprises the tips of the median covert feathers. It is fairly inconspicuous to the degree that it is often not visible in the field (Baker 1997) and will not be discussed further in

this paper. For the purposes of this study, the lower wing-bar on the six greater coverts is referred to as the wing-bar. The length of the light-colored tip of the feather is the focus of this study. Adults molt annually after breeding (Svensson 1992). Some young appear to carry the same feathers they grew in the nest right through their first breeding season, but many go through a molt of some or all their coverts in the fall (Baker 1997).

The breeding behavior of *P. humei* is described in Price and Jamdar (1991a). We conducted this study in Manali Sanctuary, Manali, Himachal Pradesh, India during the breeding seasons (May to early July) of 2001, 2003, and 2004. Typically, *P. humei* nests were found when the females were building them, incubating eggs, or raising young. Mean clutch size was 4.03 ± 0.067 S.E. ($N = 64$), and mean number of nestlings banded in a nest was 3.14 ± 0.105 S.E. ($N = 87$). We determined the sex of all individuals from the presence of a brood patch (only females incubate), behaviorally (adults), or molecularly in the lab (nestlings; Bontrager et al. submitted).

Feather collection and wing-bar measurement

The light-colored tips of the greater covert feathers, or wing-bars, are fully developed and have emerged and unfolded in nestlings by approximately ten days of age. Feathers from nestlings were collected at this time, and they were also collected from the adults, usually at the same time. We plucked the fourth greater covert feather from each wing of all birds, mounted them on microscope slides, and measured them in the laboratory. The size of the light-colored patch on each of the feathers of the wing-bar is highly correlated ($r = 0.85$, based on measurements of the second, fourth, and sixth

greater coverts on 45 adults; Marchetti 1998). Thus, the measure of the light-colored patch on the fourth greater covert feather estimates wing-bar size.

We measured the feathers under a dissecting microscope in random order (all individuals, including chicks and adults were randomized within each year). We measured from the tip of the feather, along the shaft to the point at which the first eumelanin granules could be detected alongside the shaft. The left and right feathers were measured for each individual (in mm), and these measurements were averaged. For the purposes of this paper, we refer to this measurement as “wing-bar size.”

As determined in a microsatellite paternity study, 33% ($N = 263$) of the young were not sired by their social fathers. Of these, 26 could be assigned to another male in the population, whereas 60 could not be (Bontrager et al., submitted). Only a few of these males had been sampled at the same time as the nestlings. Including these males made little difference to the analysis and they were eliminated for consistency. We found no cases where the social mother was not also the genetic mother (Bontrager et al., submitted).

Data analysis

All statistical analyses were conducted with JMP IN (SAS Institute, Cary, NC). After controlling for year, assortative mating was negligible ($r = 0.071$; $N = 85$; $P = 0.517$) and thus ignored in all analyses. In addition, we initially included fledging date (estimated as date the feathers were collected from nestlings) as a covariate in our analyses, but it had negligible effects on the estimates of parent-offspring resemblance,

and it was eliminated from our final analyses. However, there are large differences in the average size of the wing-bar in different years, and year was included as a factor in all analyses. Genetic covariances and variances were estimated by regressing offspring measurements on those of their mothers, fathers, or midparents. Only four young were recaptured in subsequent years when they were themselves breeding, and in the consequent absence of pedigrees, this method is identical to the more complicated approaches now being used (i.e., the animal model, Kruuk 2004).

We averaged the measurements for all same-sex offspring in an individual nest or, when estimating overall heritabilities, for all offspring in the nest. An estimate of the heritability was obtained using offspring on midparent regression, both without and without weighting by family size. We estimated within-sex (additive) genetic variances as twice the within-sex parent-offspring covariance. Similarly, we estimated genetic covariances as twice the between-sex parent-offspring covariance (i.e., mother-son and, separately, father-daughter). We also calculated the covariance between the sexes based on sibs, as the covariance between sex means for all nests which had both males and female chicks in our dataset. In the absence of common environmental and dominance effects, this estimates half the genetic covariance.

To obtain standard errors on the genetic estimates, we used bootstrapping, employing the program h2boot written by P. Phillips (1998). For all analyses that used h2boot, we controlled for year by standardizing the mean within each year for each sex and age class to zero.

An estimate of the cross-sex genetic correlations was calculated using the formula (Falconer and Mackay 1996, p. 316):

$$r_A = \text{cov}_{mF} / [(\text{cov}_{mM})(\text{cov}_{fF})]^{1/2} \quad (1)$$

with the subscripted letters of each covariance representing the regression of the class of individual of the first letter on that of the second. Males and females are designated by m and f, respectively, and lower case indicates nestlings, while upper case indicates adults of the given sex. We estimated genetic correlations from the mother-son covariance and father-daughter covariance separately; although in each case, the denominator is the same. An estimate of the genetic correlation from the resemblance between sibs was obtained by dividing the covariance between full sibs by the square-root of the product of the among-brood variances for each sex separately.

Results

Sex differences in wing-bar size

Table 1 shows the basic statistics for wing-bar size in each year. Mean wing-bar size differed substantially among years and between nestlings and adults, presumably reflecting different patterns of both wear and development. Wing-bars in *P. humei* are larger in nestlings than in adults (Table 1; Figure 1). This is because size of the wing-bar

in the adult is affected by wear, and the feather tips may be broken. In nestlings, however, the feathers have only just emerged, and the tips are unworn.

In nestlings in particular, wing-bar size is very similar in males and females (4.37% larger in males; effect test for sex, accounting for year: $F_{1,266} = 4.88$, $P = 0.028$). Thus, there is a slight developmental dimorphism. This may well be related to dimorphism in overall size. Wing length is not possible to measure in the nestlings, as it is far from being fully grown, but among adults in our population, males have wings 8% longer than females' (Bontrager et al. 2006, submitted).

Among adults, males had wing-bars 12.88% longer than females' (effect test for sex, accounting for year: $F_{1,199} = 11.14$, $P = 0.001$). Thus, the sex difference in adults is greater than that in the offspring. This implies that the wing-bars on the females are subject to greater wear than males, that development of the feathers in adults produces a greater dimorphism than it does in the chicks, or possibly different patterns of sex-specific mortality.

Resemblance between relatives

Nestling and offspring wing-bars could be considered different traits because the phenotypic variance in parents is more than twice as large as that in the nestlings (from Table 1; unweighted average variance across sex and years is 0.72 mm^2 in adults and 0.34 mm^2 in nestlings), and the parents' average wing-bar size is 65% the length of the nestlings'. Assuming that the genetic variance is unchanged between offspring and parent (i.e., there is no gene-environment interaction as a result of differential wear or

development of the adult feather), a regression of offspring on midparent provides an estimate of the heritability of the parental trait. Accounting for year, the regression of offspring on midparent is $b = 0.337 \pm 0.106$ S. E. ($F_{1,63} = 10.12$; $r^2 = 0.481$; $P = 0.002$). (A regression weighted by family size conducted in the program h2boot gave $b = 0.289 \pm 0.106$ S. E.) Within-sex single-parent offspring regressions using h2boot gives similar estimates of heritability; 0.367 ± 0.172 S. E. for daughter on mother ($N = 71$) and 0.340 ± 0.168 S. E. for son on father ($N = 46$). A heritability of 0.34 implies that 34% of the phenotypic variance in the adults is genetic, and given that the estimate of phenotypic variance is 2.13 times higher in adults than chicks, this implies that 72% of the phenotypic variance in the offspring is genetic. These calculations ignore the possibility that some of the shared resemblance among relatives is due to environmental factors.

In Table 2, we show the bootstrapped covariances, having first corrected for differences between years. Each covariance has been multiplied by two in the table so that the estimate can be equated with the genetic (co)variance. Within-sex covariances are very similar for each sex, and both are significantly different from 0 ($P < 0.05$). However, the covariance between father and daughter is almost twice that between mother and son, and the difference is significant (two-tailed t -test, using the standard errors in Table 2, $t_{122} = 2.08$, $P < 0.05$). The large difference in the cross-sex covariances may imply maternal or other environmental effects (see discussion), making calculations of genetic correlations suspect. However, application of the standard formula (equation 1) gives genetic correlations of 1.30 (from the father-daughter regression) and 0.76 (from the mother-son regression), when based on the regressions using JMP. The same

correlations are estimated as 1.57 (95% confidence limits 0.3-6.5) and 0.83 (95% confidence limits 0.07-3.6), respectively, from the bootstrapped datasets.

Using JMP, the phenotypic correlation between brood means for all broods that had members of each sex (full sibs only) was, after controlling for year, $r = 0.471$, $N = 33$ broods, $P = 0.006$. The corresponding covariance is 0.018 mm^2 . In a nested ANOVA, the fraction of the variance among broods for females (nested within year) is estimated as 0.58 for females ($F_{53,41} = 5.15$, $P < 0.001$) and 0.22 for males ($F_{48,31} = 2.68$, $P = 0.002$). From these values, we obtain an estimate of the genetic correlation between the sexes as 0.86, although as in the other measures of resemblance, this is affected to an unknown extent by environmental effects (e.g., common nest environment).

Discussion

With respect to parent-offspring resemblance, twice the average cross-covariance between the sexes (0.042 mm^2) is actually slightly higher than twice the average within-sex covariance (0.035 mm^2), implying a high genetic correlation between the sexes for the size of the wing-bar. The estimate of the genetic correlation has broad confidence limits, but is significantly different from 0, and not significantly different from 1.0. These results suggest that evolution of sexual dimorphism in wing-bar size may be highly constrained, with selection on one sex leading to strong correlated evolution in the other (Lande 1980). The slight difference in mean size of the wing-bar (Figure 1) may reflect the difference in wing length between the sexes (8% in adults). Badyaev (2002) has argued that sexual dimorphism in size may be rather easily achieved by altering growth

trajectories of the two sexes, even if such sex-limited variation accounts for only a small component of the total genetic variation in overall size.

Previous studies of genetic correlations between the sexes in birds are few. Most of those conducted for morphological traits (van Noordwijk et al. 1980; Price 1984; Merilä et al. 1998) have found a positive and high genetic correlation (i.e., close to 1) between the sexes, albeit with large standard errors.

In general, many socially and sexually selected traits are present only in adults, making data for field studies difficult to collect because they depend on returns of offspring. Any selection acting before birds are recaptured complicates heritability estimates, and large sample sizes are difficult to obtain. This was part of our original motivation in studying *P. humei*. In this species, nestlings have fully developed wing-bars while still in the nest, allowing the trait to be measured on both parents and chicks in a single season.

However, some genetic correlations for color traits in other bird species have been estimated. In two captive breeding studies, estimates of the genetic correlation between the sexes in the sexually selected bill color of zebra finches, *Taeniopygia guttata* come out to be 0.91 (Price and Burley 1993), and after a cross-fostering experiment, 0.81 (Price 1996). Other studies have presented estimates of phenotypic correlations across generations between the sexes (i.e., the father-daughter correlation or mother-son correlation) for tail length in the barn swallow, *Hirundo rustica* (Møller 1993, 1994, p. 288) and for plumage coloration and for degree of spotting in the barn owl, *Tyto alba*, but in neither case is it clear that these correlations were translated into the genetic correlation.

Despite our inference of a high genetic correlation between the sexes, the conclusion might be suspect due to the finding of a significantly higher covariance between the father and daughter than the mother and son. It is not uncommon in estimates of genetic correlations to find that the correlation between trait X in the parents and trait Y in the offspring differs from the correlation between trait Y in the parents and trait X in the offspring (Lande and Price 1989). Lande and Price (1989) suggested that this pattern could result either from differential selection on offspring or maternal effects. In this study, we can most likely eliminate differential selection because very few individual offspring in any brood died prior to measurement. Thus, the best explanation for the different cross-covariances is a sex-specific maternal effect. Because the regression of son on mother is lower than that of daughter on father, the implication is that mothers with large wing-bars are negatively impacting the development of the wing-bar in their sons. (This conclusion must be qualified because the within-sex covariances are not significantly different from the son-mother covariance, so it is possible that any maternal effect is not sex-specific.) The presence of a negative maternal effect implies that the estimated genetic correlation obtained from the mother-son regression is too low, supporting the thesis that the genetic correlation is high.

The tip of the feather is the first part to form and lies encased in a waxy sheath. The sheaths are well developed within three days of hatching, so factors affecting the development of the wing-bar must operate prior to this time, probably while still in the egg. In house finches, Young and Badyaev (2004) and Badyaev et al. (2005) have found evidence for strong sex-specific maternal effects during egg development, with oocytes destined to produce males growing up to five times faster and ovulating sooner than those

destined to produce females (Young and Badyaev 2004). Such effects could interact with maternal phenotype to influence both hormonal (Badyaev et al. 2005) and nutrient content of eggs, and hence development of males. Badyaev (2005a) has argued that such sex-specific maternal effects are common because they allow a rapid way to respond to novel environmental challenges. Thus, the results of our study support the idea that the wing-bar is genetically constrained, but potentially, dimorphism could rapidly arise as a result of modifications of sex-specific maternal effects.

Although a high genetic correlation may account for the similar expression of the wing-bar in males and females, we suggest that the expression of wing-bar in female *P. humei* is unlikely to be strongly maladaptive. Females flick their wings exhibiting the wing-bars in several social contexts, including social selection in the non-breeding season. In particular, both sexes hold territories in the winter (Gross and Price 2000), and the wing-bar is likely to be displayed by both males and females in establishing and maintaining winter territories. Wing-bars may also function in within-sex dominance interactions during the breeding season, as a focus of male choice (reviewed in Amundsen 2000; Amundsen and Pärn 2006). They may also contribute to prey-flushing during foraging (Jablonski 1996; but see Marchetti and Price 1997).

In conclusion, we find evidence that the similarity in the form of the wing-bar in the two sexes results from a high genetic correlation between the sexes, but suggest that the relatively small dimorphism in wing-bar size is a consequence of selection pressures on females for its maintenance. Even if dimorphism in wing-bar size is predicted to be difficult to evolve genetically, sex-specific maternal effects imply that dimorphism could

be fairly easily modified developmentally, and this could set the stage for subsequent genetic evolution.

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The text of Chapter II, in full, is a reprint of the material as it has been submitted to the journal *Evolution*. I was the primary author of this chapter. Trevor Price directed and supervised the research in this chapter.

Bontrager, A.L. and T. D. Price. (submitted). Genetic correlation between the sexes in the color pattern of a sexually monomorphic bird. *Evolution*.

Table III.1. Mean wing-bar size values by age and sex for each year.

Group	Year	Mean wing-bar size (mm)	Standard deviation	N
Adult female	2001	1.009	0.272	30
Adult male	2001	1.223	0.331	33
Nestling female	2001	1.877	0.299	55
Nestling male	2001	1.990	0.182	39
Adult female	2003	1.408	0.340	35
Adult male	2003	1.635	0.358	38
Nestling female	2003	2.285	0.257	60
Nestling male	2003	2.321	0.25	50
Adult female	2004	1.523	0.422	28
Adult male	2004	1.544	0.341	28
Nestling female	2004	2.155	0.207	31
Nestling male	2004	2.248	0.211	34

Table III.2. Covariances between relatives based on 1000 bootstrapped samples.*

Relationship	Standard error		N	Genetic correlation ¹
	Estimate (mm ²)	(mm ²)		
Daughter-mother	0.035	0.016	73	0.83 1.57
Son-mother	0.029	0.014	71	
Daughter-father	0.055	0.022	53	
Son-father	0.035	0.021	49	

*All covariances and standard errors have been doubled, so that, in the absence of environmental effects these can be considered estimates of genetic variances and covariances.

¹Computed assuming the covariances are all a result of genetics and there are no environmental covariances.

Genetic variances were calculated as the geometric mean of the two within-sex covariances.

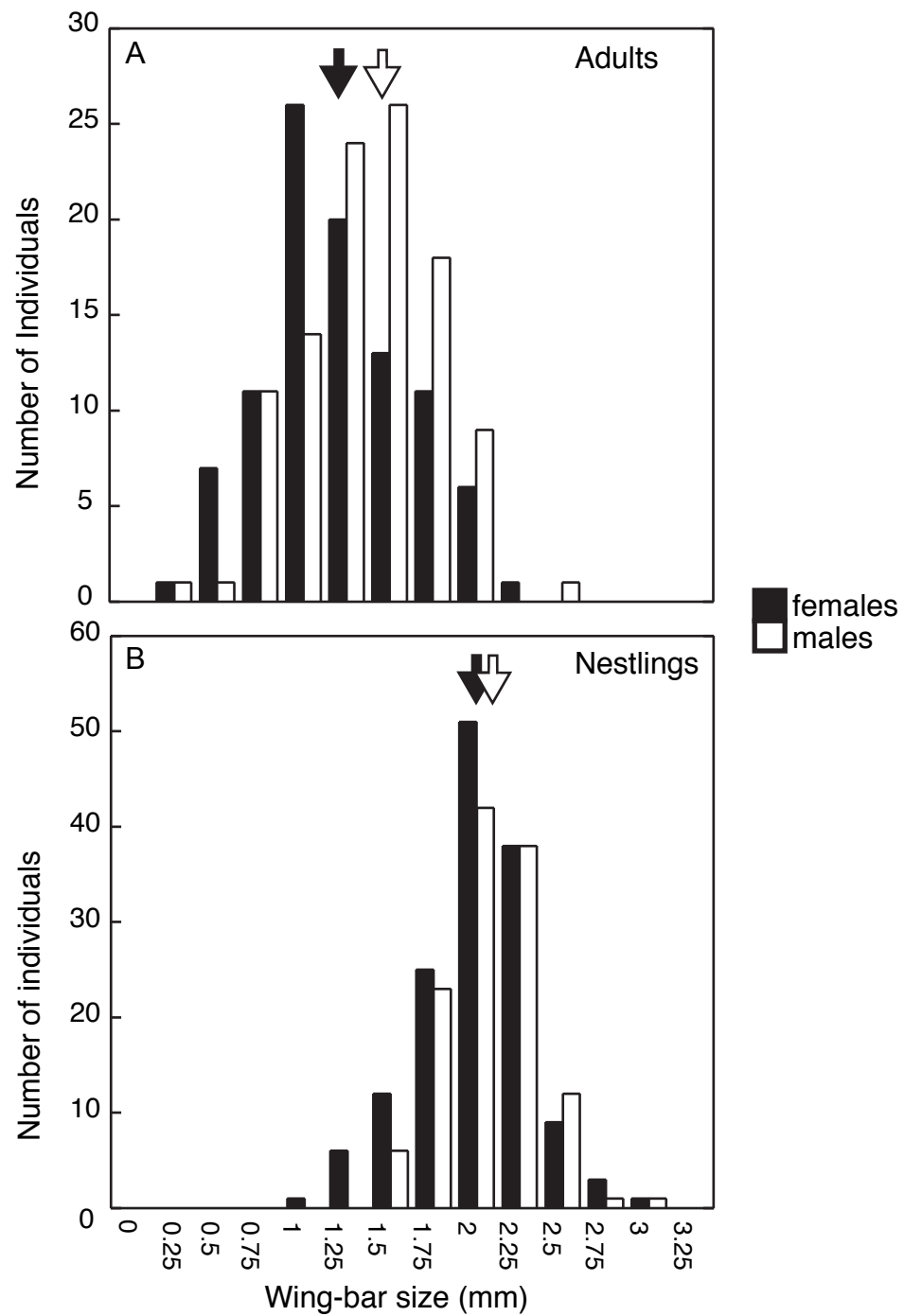


Figure III.1. Distribution of wing-bar sizes by sex in (A) adult and (B) nestling *P. humei*. Arrows indicate mean wing-bar size values for each sex.

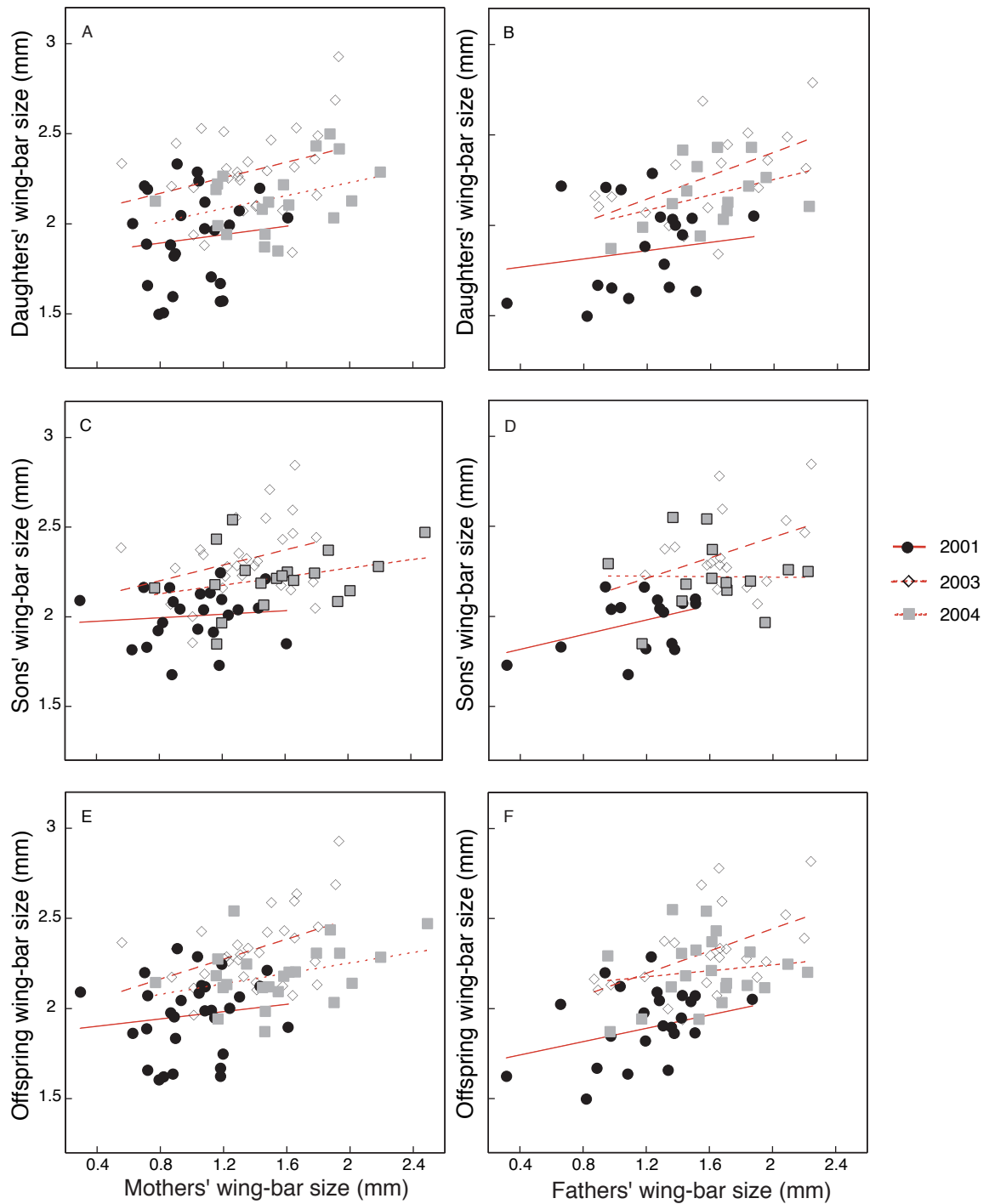


Figure III.2. Scatter plots of parent-offspring regressions of wing-bar size. Offspring data are mean values per nest. (A) Daughter on mother, (B) Daughter on father, (C) Son on mother, (D) Son on father, (E) Offspring on mother, (F) Offspring on father.

Literature Cited

- Amundsen, T. 2000. Why are female birds ornamented? *Trends Ecol. Evol.* 15:149-155.
- Amundsen, T., and H. Pärn. 2006. Female coloration: review of functional and nonfunctional hypotheses. Pp. 280-345 in G. E. Hill and K. J. McGraw, ed. *Bird coloration vol 2: function and evolution*. Harvard Univ. Press, Cambridge, MA.
- Badyaev, A. V. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol. Evol.* 17:369-378.
- Badyaev, A. V. 2005a. Maternal inheritance and rapid evolution of sexual size dimorphism: passive effects or active strategies? *Am. Nat.* 166:S17-S30.
- . 2005b. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. *Proc. R. Soc. Lond. B.* 272:877-886.
- Badyaev, A. V., H. Schwabl, R. L. Young, R. A. Duckworth, K. J. Navarra, and A. F. Parlow. 2005. Adaptive sex differences in growth of pre-ovulation oocytes in a passerine bird. *Proc. R. Soc. Lond. B.* 272:2165-2172.
- Baker, K. 1997. *Warblers of Europe, Asia and North Africa*. Princeton Univ. Press, Princeton.
- Bontrager, A. L., Gibbs, H. L., Price, T. D. 2006. High incidence of extra-pair paternity in a monochromatic, socially monogamous passerine *Phylloscopus humei*, based on newly identified microsatellite markers. *Mol. Ecol.* submitted.
- Eaton, M. D. 2005. Human vision fails to distinguish widespread sexual dichromatism among sexually "monochromatic" birds. *Proc. Natl. Acad. Sci. USA.* 102:10942-10946.
- Falconer, D. S., and T. F. C. Mackay. 1996. *Introduction to Quantitative Genetics*. Longman, Essex.
- Figuerola, J., and A. J. Green. 2000. The evolution of sexual dimorphism in relation to mating patterns, cavity nesting, insularity and sympatry in the Anseriformes. *Functional Ecol.* 14:701-710.
- Fisher, R. A. 1958. *The genetical theory of natural selection*. Dover Publ., N.Y.
- Gross, S. J., and T. D. Price. 2000. Determinants of the northern and southern range limits of a warbler. *J. Biogeogr.* 27:869-878.

- Jablonski, P. 1996. Dark habitats and bright birds: Warblers may use wing patches to flush prey. *Oikos* 75 : 350-352.
- Kruuk, L. E. B. 2004. Estimating genetic parameters in natural populations using the 'animal model'. *Phil. Trans. R. Soc. of Lond.B.* 359:873-890.
- Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292-305.
- Lande, R., and T. Price. 1989. Genetic correlations and maternal effect coefficients obtained from offspring-parent regression. *Genetics* 122:915-922.
- Marchetti, K. 1993. Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* 362:149-152.
- . 1998. The evolution of multiple male traits in the yellow-browed leaf warbler. *Anim. Behav.* 55:361-376.
- Marchetti, K., and T. Price. 1997. The adaptive significance of colour patterns in the Old World leaf warblers, genus *Phylloscopus* . *Oikos*. 79: 410-412
- Merilä, J., B. C. Sheldon, and H. Ellegren. 1998. Quantitative genetics of sexual size dimorphism in the collared flycatcher, *Ficedula albicollis*. *Evolution* 52:870-876.
- Møller, A. P. 1993. Sexual selection in the barn swallow *Hirundo rustica*. 3. Female tail ornaments. *Evolution* 47:417-431.
- Møller, A. P. 1994. Sexual selection and the barn swallow. Oxford Univ. Press, New York.
- Peterson, A. T. 1996. Geographical variation in sexual dichromatism in birds. *Bull. Brit. Orn. Club* 116:156-172.
- Phillips, P. C. 1998. H2boot: bootstrap estimates and test of quantitative genetic data. Univ. of Oregon. Software available at www.uoregon.edu/~pphil/software.html.
- Price, D. K. 1996. Sexual selection, selection load and quantitative genetics of zebra finch bill colour. *Proc. R. Soc. of Lond. B.* 263:217-221.
- Price, D. K., and N. T. Burley. 1993. Constraints on the evolution of attractive traits - genetic (co)variance of zebra finch bill color. *Heredity* 71:405-412.
- Price, T. D. 1984. The evolution of sexual size dimorphism in Darwin Finches. *Am. Nat.* 123:500-518.

- Price, T. 2002. Domesticated birds as a model for the genetics of speciation by sexual selection. *Genetica*. 116:311-327.
- Price, T., and G. L. Birch. 1996. Repeated evolution of sexual color dimorphism in passerine birds. *Auk* 113:842-848.
- Price, T., and N. Jamdar. 1991a. Breeding biology of the yellowbrowed leaf warbler *Phylloscopus inornatus* in Kashmir. *J. Bombay Nat. Hist. Soc.* 88:1-16.
- . 1991b. Breeding of eight sympatric species of *Phylloscopus* warblers in Kashmir. *J. Bombay Nat. Hist. Soc.* 88:242-255.
- Price, T., and T. Pavelka. 1996. Evolution of a colour pattern: history, development, and selection. *J. Ecol. Biol.* 9:451-470.
- Rasmussen, P. C., and J. C. Anderton. 2005. *Birds of South Asia: The Ripley Guide*. Lynx Edicions, Barcelona.
- Reeve, J. P., and D. J. Fairbairn. 2001. Predicting the evolution of sexual size dimorphism. *J. Evol. Biol.* 14:244-254.
- Roulin, A., C. Dijkstra, C. Riols, and A. L. Ducrest. 2001. Female- and male-specific signals of quality in the barn owl. *J. Evol. Biol.* 14:255-266.
- Svensson, L. 1992. *Identification guide to European passerines*, Stockholm.
- Young R.L., and Badyaev A.V. 2004. Evolution of sex-biased maternal effects in birds: I. Sex-specific resource allocation among simultaneously growing oocytes. *J. Evol. Biol.* 17:1355-1366.