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An experimental test for alternative reproductive strategies underlying a female-limited polymorphism

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Abstract

Polymorphism often corresponds to alternative mating tactics in males, but much less is known about this relationship in females. However, recent work suggests that selection for alternative reproductive strategies in females can maintain genetic variation in important life-history traits. Brown anole lizards (*Anolis sagrei*) exhibit a genetically based polymorphism in dorsal pattern that is expressed only by females, which occur in bar (B), diamond (D) and intermediate diamond–bar (DB) morphs. Here, we use a combination of natural history data, captive breeding studies and phenotypic manipulations of reproductive investment to test the hypothesis that this polymorphism corresponds to morph-specific patterns of reproductive investment. Three years of data from wild females and two generations of captive breeding revealed no differences among morphs in the frequency of egg production or in the number, frequency, size or sex ratio of offspring. Manipulations of reproductive investment via surgical ovariectomy revealed significant costs of reproduction with respect to survival, growth, immune function and haematocrit, but the magnitudes of these costs did not differ among morphs. Collectively, our results refute the hypothesis that this sex-limited polymorphism is maintained by selection for alternative reproductive strategies. We compare this finding to other systems in which polymorphic females exhibit alternative reproductive tactics and discuss other selective factors that could maintain polymorphism in anoles.

Introduction

Species that invest heavily in reproduction generally exhibit early maturation and low adult survival, whereas species with low reproductive investment typically delay maturation and have high adult survival (Stearns, 1992; Roff, 2002). Despite these broad generalities, individual populations can exhibit considerable phenotypic and genetic variation in life-history strategies (Sinervo *et al.*, 2000; Mappes & Koskela, 2004). This is somewhat counterintuitive, given that selection is expected to rapidly deplete additive genetic variation for life-history traits, which are tightly linked to fitness (Price & Schluter, 1991; McCleery *et al.*, 2004). However, theoretical and empirical studies suggest that alternative life-history strategies can be maintained by frequency- or density-dependent selection on females (Roughgarden, 1971; Sinervo *et al.*, 2000; Sinervo, 2001; Mappes *et al.*, 2008; Vercken *et al.*, 2010), similar to the maintenance of alternative mating tactics in males (Shuster & Wade, 1991; Ryan *et al.*, 1992; Sinervo & Lively, 1996; Sinervo *et al.*, 2007). Alternative reproductive strategies in females may therefore have similar implications for the maintenance of genetic variation, the evolution of polymorphism and the role of polymorphism in speciation (Gray & McKinnon, 2006; Svensson *et al.*, 2009; Corl *et al.*, 2010).

Much of our understanding of alternative reproductive tactics is attributable to the study of genetically based polymorphisms (Shuster & Wade, 1991; Ryan *et al.*, 1992; Lank *et al.*, 1995; Sinervo & Lively, 1996). Polymorphisms provide tractable systems because they partition complex phenotypic and genetic variation into

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discrete classes, thus simplifying the study of evolutionary dynamics in the wild (Calsbeek *et al.*, 2010a). The association between polymorphism and alternative reproductive tactics has been studied extensively in males and in taxa as diverse as isopods, molluscs, insects and vertebrates (reviewed by Oliveira *et al.*, 2008). However, alternative reproductive strategies have received considerably less attention in females (Hanson & Warner, 1997; Roulin *et al.*, 2003; Vercken *et al.*, 2007; Svensson *et al.*, 2009).

Although alternative female reproductive strategies and female-limited polymorphisms have been studied in mammals, birds and insects (e.g. Roulin *et al.*, 2003; Mappes *et al.*, 2008; Svensson *et al.*, 2009), two of the clearest examples have recently been described in lizards. Female side-blotched lizards (*Uta stansburiana*) occur in both orange and yellow throat-colour morphs. Orange-throated females lay large clutches of small eggs and are favoured at low population density, whereas yellow-throated females produce small clutches of large eggs and are favoured at high density (Sinervo *et al.*, 2000; Sinervo, 2001). Female common lizards (*Zootoca* [*Lacerta*] *vivipara*) occur in three ventral colour morphs that differ in their reaction norms for clutch size, hatching success and progeny sex ratios across various environmental and phenotypic gradients (Vercken *et al.*, 2007, 2010). In each species, colour morphs also differ in correlated suites of behaviours, morphologies and physiologies that accompany their distinct reproductive strategies (Svensson *et al.*, 2001, 2002; Lancaster *et al.*, 2007, 2010; Vercken & Clobert, 2008).

Identifying additional polymorphisms is an important endeavour if we are to expand our understanding of alternative female reproductive tactics beyond these few model systems. In this study, we examine the patterns of reproductive investment underlying a sex-limited polymorphism in females of a third, distantly related lizard species, the brown anole (*Anolis sagrei*). Among vertebrates, anoles comprise one of the few examples of a colour polymorphism that occurs only within females (Schoener & Schoener, 1976). On the Great Bahamas Bank, female brown anoles exhibit three distinct dorsal patterns classified as B, D and DB (Fig. 1). This polymorphism has a strong genetic basis (Calsbeek *et al.*, 2008, 2010b), and several lines of evidence suggest that morphs may differ in trade-offs associated with reproduction. First, viability selection during the reproductive season favours low immune response in B morphs and high immune response in D morphs (Calsbeek *et al.*, 2008), and subsequent experiments have directly linked both survival and immune function to reproductive investment (Cox & Calsbeek, 2010b; Cox *et al.*, in press). Second, morph frequencies and life-history patterns covary geographically across two major islands on the

Fig. 1 Three alternative dorsal patterns expressed by *Anolis sagrei* females on the Great Bahamas Bank. L–R: bar (B), diamond–bar (DB) and diamond (D).

Great Bahamas Bank. At our study site on Great Exuma, the B morph is rare, annual survival is low and reproductive investment is high, whereas on Eleuthera, the D morph is rare, annual survival is higher and annual reproductive investment is relatively low (Calsbeek *et al.*, 2010b; Cox & Calsbeek, 2010a). Finally, natural selection acts differentially on morphs with respect to variation in the density of neighbouring females, such that B females have a survival advantage at low density, whereas DB and D females have a survival advantage at high density (Calsbeek *et al.*, 2008, 2010b). This pattern is analogous to other systems in which females exhibit alternative reproductive strategies (Sinervo *et al.*, 2000; Mappes *et al.*, 2008; Vercken *et al.*, 2010). However, the mechanisms that drive these apparent patterns of morph-specific selection are currently unclear and the hypothesis that female morphs express alternative reproductive strategies remains untested.

A major difficulty in assessing reproductive trade-offs is that individual variation in reproduction and survival is often correlated with other confounding factors, such as age, size, physical condition or habitat quality. One way around this limitation is to directly manipulate reproductive investment while randomizing or controlling for confounding variation (Landwer, 1994; Koivula *et al.*, 2003; Cox, 2006). Such phenotypic manipulations have proven particularly useful in elucidating the physiological basis of alternative reproductive strategies (Sinervo *et al.*, 2000; Svensson *et al.*, 2002; Lancaster *et al.*, 2007). We have previously shown that the elimination of reproductive investment in brown anoles results in dramatic increases in survival, growth, immune function and haematocrit (Cox & Calsbeek, 2010b; Cox *et al.*, in press). Here, we extend this experimental paradigm to test the hypothesis that female morphs differ in the extent to which reproductive investment impacts these important fitness-related traits. We also use 3 years of descriptive natural history data and two generations of captive breeding studies to test for morph differences in the frequency of egg production and in the number, frequency, size and sex ratio of their progeny. On the basis of our previous studies, we predicted that B females should exhibit characteristics associated with high annual survival and low fecundity (e.g. low reproductive frequency, large offspring size, reduced costs of reproduction), whereas D females should exhibit alternative traits associated with low annual survival and high fecundity (e.g. high reproductive frequency, small offspring size, larger costs of reproduction).

Material and methods

Study species and classification of morphs

The brown anole (*Anolis sagrei*) is a small, semi-arboreal lizard that is native to islands throughout the West Indies. We studied a wild population of brown anoles at February Point, near Georgetown on the island of Great Exuma, The Bahamas (23°29′N, 75°45′W). Further details on this study site and the reproductive biology of females in this population are provided in previous publications (Cox & Calsbeek, 2010b; Cox *et al.*, in press). Females from this population exhibit a genetically based polymorphism in dorsal pattern that is present at hatching and invariable throughout ontogeny (Fig. 1). This polymorphism is not expressed in males, which are uniformly grey or brown and lack conspicuous dorsal patterning as adults.

We classified the dorsal pattern of each female as B, D or DB using criteria described in previous studies (Calsbeek *et al.*, 2008, 2010b). Briefly, we classified females as B based on the presence of an unbroken longitudinal bar and as D based on the presence of an uninterrupted diamond pattern (Fig. 1). Females with misshapen diamonds, interrupted bars or a combination of diamond and bar patterns were classified as DB. We note that, although variation in dorsal pattern is low within the B and D morphs, variation within the DB morph is considerable, such that our discrete classification may obscure underlying phenotypic and genetic variation. However, comparable phenotypic variation is often observed within morphs in polymorphic systems, including anoles (Schoener & Schoener, 1976; Vercken *et al.*, 2008). For simplicity and comparison with previous studies (Calsbeek *et al.*, 2008, 2010b), we employed a trimorphic classification scheme.

Egg production in the wild

Brown anoles iteratively lay single-egg clutches at approximately 1- to 2-week intervals. This occurs because only one follicle per ovary matures at a given time and the cycle of follicular maturation and ovulation is offset between right and left ovaries. However, the ovulation of a follicle from one ovary often precedes the oviposition of the fertilized egg from the opposite follicle, such that females may contain up to two oviductal eggs at any time. Because brown anoles repeatedly lay single-egg clutches at frequent intervals throughout a lengthy reproductive season (April–October; Lee *et al.*, 1989), the number of eggs present in a female at any given time does not provide useful information on her net annual reproductive effort. However, across a large cross-sectional sample, the relative proportions of individuals with zero, one or two eggs can provide information about relative reproductive effort in a given species or population (Andrews & Rand, 1974).

Over three consecutive years (2007–2009), we performed surgical laparotomies on a total of 1320 wild females ($n = 304$ B, 616 DB, 400 D). For each female, we recorded the number of oviductal eggs present at the time of surgery (May), which was near the peak of the reproductive season (Lee *et al.*, 1989). We tested for morph differences in reproductive effort by comparing the number of eggs (0, 1, 2) as a function of morph (fixed effect: B, DB, D), year (random effect: 2007, 2008, 2009) and snout-vent length (SVL, nearest mm) using ordinal logistic regression. We also included the interaction between morph and SVL to test for morph differences in size-specific fecundity. All statistical analyses, here and elsewhere, were conducted in JMP (version 6.0.2; SAS Institute Inc., Cary, NC, USA).

Offspring production in captivity

Because the iterative production of single-egg clutches complicates the measures of annual reproductive effort in the wild, we also quantified the production of offspring by females maintained in a captive breeding colony. In June 2007, we captured 69 females after they had mated in the wild and returned them to our captive breeding facility. We allowed these females to oviposit in potted plants and then collected and raised their progeny to adulthood following methods reported elsewhere (Cox *et al.*, 2009). When these progeny were sexually mature, we allowed each daughter to mate with two different males and collected a second generation of progeny (Cox & Calsbeek, 2010b). Although anoles lay single-egg clutches, they can store sperm and produce viable progeny for up to 4 months following mating (Calsbeek *et al.*, 2007; Calsbeek & Bonneaud, 2008), allowing us to measure reproductive effort for several months following mating. To minimize confounds between fecundity and sperm limitation, we omitted females that produced one or fewer offspring when quantifying offspring production. We also quantified reproductive frequency as the average time between successive progeny for each female that produced more than one offspring. This measure is robust to any variance because of sperm limitation because it estimates reproductive effort over an interval when females are known to be capable of producing fertile eggs. As an additional component of reproductive investment, we measured the average mass at hatching of all progeny produced by each female. Finally, we measured the sex ratio (proportion of sons to total offspring) produced by each female because, although the sexes are similarly sized at hatching (Cox *et al.*, 2009), males grow more quickly than females immediately after hatching and may therefore reflect greater maternal investment.

We collected progeny from a total of 134 captive females ($n = 30$ B, 58 DB, 46 D), although 26 females produced only one offspring and were not included in the analyses of fecundity or the frequency of reproduction. We tested for morph differences in the number of progeny using a generalized linear model (GLM) with Poisson error distributions and log link functions. We tested for morph differences in reproductive frequency using analysis of variance (ANOVA). We tested for morph differences in hatchling mass using ANOVA with the average hatch mass of each female's progeny weighted by her total number of offspring. We tested for morph differences in progeny sex ratio using GLM with binomial error distributions and logit link functions, weighting estimates of sex ratio by the total number of offspring contributing to each estimate. For each analysis of morph effects, we also included maternal size (SVL or mass) and morph × maternal size interactions to account for any effects of maternal size on reproductive investment. We also examined morph differences in the potential trade-off between offspring quantity and quality by testing for differences in the slope of the relationship between number of progeny and the average mass of progeny among morphs.

We did not detect any differences between the initial sample of wild-caught females and their captive-bred daughters with respect to number of offspring (GLM: $\chi^2 = 0.21$, d.f. = 1; $N = 108$; $P = 0.65$), reproductive frequency (ANOVA: $F_{1,94} = 1.81$, $F = 0.18$) or sex ratio (GLM: $\chi^2 = 0.93$, d.f. = 1; $N = 134$; $P = 0.34$), although hatch mass was significantly lower for the second generation of captive-bred progeny (ANOVA: $F_{1,129} = 33.01$; $F < 0.0001$). We therefore included an effect of generation in all analyses of progeny hatch mass.

Experimentally assessing costs of reproduction

We manipulated reproductive investment by surgically ovariectomizing (OVX) wild females near the onset of the breeding season (May) in each of three separate years (2007–2009). A second group of females received sham (SHAM) surgeries to control for surgical effects while leaving their reproductive functions intact. All females were sexually mature (SVL > 38 mm) at the time of surgery. These surgical procedures are discussed in detail elsewhere (Cox & Calsbeek, 2010b) and resulted in a total of 297 OVX females (87 B, 112 DB, 98 D) and 293 SHAM females (88 B, 105 DB, 100 D). Following surgery, we released females to their exact sites of capture and left them undisturbed until the end of the breeding season (September, 4 months post-treatment), at which point we attempted to recapture every surviving lizard. Elsewhere, we have used Cormack–Jolly–Seber mark–recapture models to show that, although recapture success slightly underestimates actual survival in this population, overall treatment effects on survival are robust to this error (Cox & Calsbeek, 2010b). We measured growth based on change in SVL (nearest 1 mm) and body mass (nearest 0.1 g) from premanipulation (May) to recapture (September). We have previously inferred costs of reproduction from these experiments by demonstrating that OVX exceed SHAM with respect to both survival and growth (Cox & Calsbeek, 2010b). To determine whether these costs of reproduction differed among morphs, we tested for statistical interactions between treatment (fixed effect: OVX, SHAM) and morph (fixed effect: B, DB, D) using models that also included terms for year (random effect: 2007, 2008, 2009) and, in the case of growth data, initial size (SVL or mass). We analysed survival (live, die) using logistic regression and growth using linear mixed models (LMM).

For those females recaptured at the end of the 2009 experiment, we also measured three physiological parameters that are impacted by reproductive investment: immune function, haematocrit and parasitaemia. We measured immune function using the phytohemagglutinin (PHA) skin-swelling technique, an *in vivo* assay that is commonly used in wild vertebrates, including lizards (Svensson *et al.*, 2001; Oppliger *et al.*, 2004; Calsbeek *et al.*, 2008). Injection of PHA induces the influx of lymphocytes, heterophils, thrombocytes, basophils and macrophages, which collectively manifest as localized swelling at the site of injection (Martin *et al.*, 2006). We measured haematocrit as the proportion of blood cells to total blood volume for samples centrifuged at 1300 *g* in microhaematocrit tubes. We measured parasitaemia by preparing blood smears from each female and counting the number of parasitized cells per 10^3 red blood cells using standard methods (Cox *et al.*, in press). We tentatively identified most blood parasites as *Plasmodium azurophilum,* although *P. floridense* and other apicomplexans are observed in Caribbean anoles and may have contributed to our counts (Schall, 1992; Staats & Schall, 1996). Further details on our immune, haematocrit, and parasitaemia assays are provided elsewhere (Cox *et al.*, in press). We tested whether morphs differed in the extent to which reproduction impacted these three physiological parameters by testing for morph × treatment interactions using ANOVA. Because measures of parasitaemia were not normally distributed, we used $\log_{10}$-transformed measures of parasitaemia or nonparametric Wilcoxon tests for these analyses.

Natural variation in survival, growth and physiology

Inferences from the experiments described earlier are derived from morph × treatment interactions and address the question of whether morphs differ in the relative magnitude of the various costs associated with reproduction. However, it is also relevant to ask whether reproductive females of each morph naturally differ in survival, growth, immune function, haematocrit and parasitaemia, as would be predicted if they differ in reproductive investment. We therefore tested for morph differences in each of these response variables within the SHAM group of reproductive females using logistic regression (survival data) or LMM with morph as a fixed effect, year as a random effect and size as a covariate (growth analyses only). We also examined morph differences in potential trade-offs between growth, immune function and $\log_{10}$-parasitaemia by testing for morph differences in the slopes of the relationships between pairwise combinations of these physiological traits. The number of SHAM females for which we measured both haematocrit and other physiological traits was generally too small to support meaningful tests for separate slopes within morphs.

Corrections for multiple testing

We conducted three separate studies (egg production in the wild, offspring production in captivity, experimental manipulations in the wild), each of which was replicated in more than 1 year. Moreover, we often measured multiple response variables in a given study, such that each response could be viewed as an additional test of the same hypothesis that morphs differ in reproductive strategies. In these situations, we present corrections to the experiment-wide Type I error rate (α') using the Dunn–Sidak method:

$$\alpha' = 1 - (1 - \alpha)^{1/k}$$

where $\alpha = 0.05$, the conventional level for Type I error, and k = the number of tests being compared (Gotelli & Ellison, 2004). Although we do not favour one statistical viewpoint over another on the issue of multiple testing, we nonetheless discuss significant results in the light of these corrections, when appropriate.

Results

Egg production in the wild

Overall, 14% of females had two oviductial eggs, 61% had one egg and 25% had zero eggs when sampled in May, near the peak of the breeding season (Fig. 2). Number of eggs did not differ among morphs (logistic:

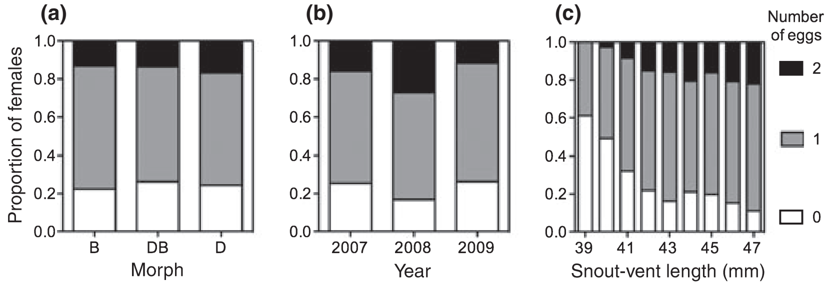

Fig. 2 The proportion of wild females with zero, one or two oviductal eggs as a function of (a) morph, (b) year and (c) snout-vent length.

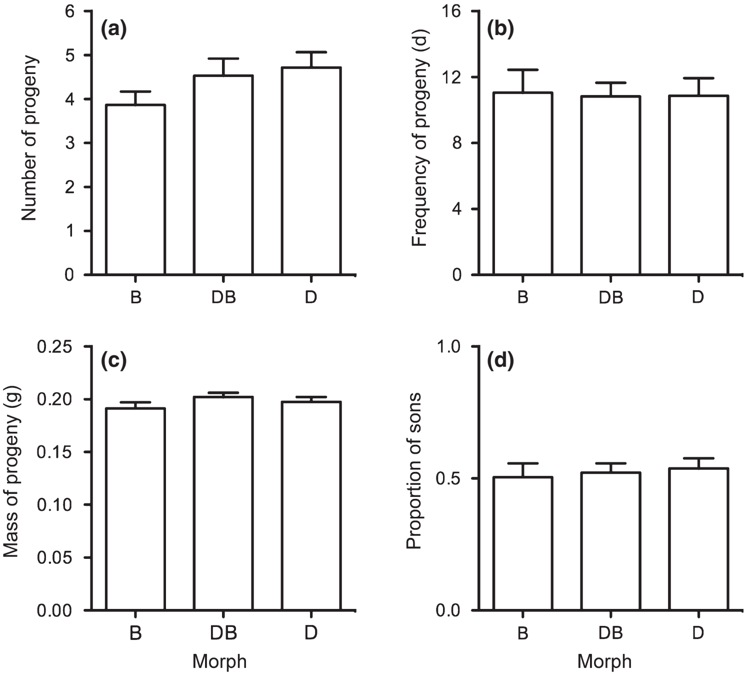

Fig. 3 Female morphs did not differ in (a) number of offspring, (b) frequency of offspring, (c) mass of offspring at hatching or (d) sex ratio of progeny. Data are means (± 1 SE) with the exception of panel c, which reports least-squares means (± 1 SE) from analyses controlling for differences among generations (see text).

$\chi^2 = 0.33$, d.f. = 2; $N = 1320$; $P = 0.85$; Fig. 2a). A greater proportion of females had two eggs (and a smaller proportion had zero eggs) in 2008 relative to 2007 or 2009 (logistic: $\chi^2 = 39.67$, d.f. = 2; $N = 1320$; $P < 0.0001$; Fig. 2b). Number of oviductal eggs increased with body size (logistic: $\chi^2 = 61.18$, d.f. = 1; $N = 1320$; $P < 0.0001$; Fig. 2c), but this allometry was similar across morphs (logistic; morph × SVL: $\chi^2_2 = 3.08$; $N = 1320$; $P = 0.21$). Morphs did not differ in body size (ANOVA: SVL: $F_{2,1318} = 1.69$; $P = 0.18$; body mass: $F_{2,1318} = 1.79$; $P = 0.17$).

Offspring production in captivity

The average number of offspring produced by each female did not differ among morphs (GLM: $\chi^2 = 2.14$, d.f. = 2; $N = 108$; $P = 0.34$; Fig. 3a). Production of offspring increased weakly with maternal SVL (GLM: $\chi^2 = 2.75$, d.f. = 1; $N = 108$; $P = 0.10$), but this allometry did not differ among morphs (GLM; morph × SVL: $\chi^2 = 0.57$, d.f. = 2; $N = 108$; $P = 0.75$). On average, females produced one offspring every 10.9 days, but this frequency did not differ among morphs (ANOVA: $F_{2,89} = 0.02$; $P = 0.98$; Fig. 3b). Frequency of reproduction was also unrelated to either maternal SVL (ANOVA: $F_{1,89} = 0.44$; $P = 0.51$) or the interaction of morph × SVL (ANOVA: $F_{2,89} = 0.23$; $P = 0.79$). The average mass of progeny at hatching did not differ as a function of maternal morph (ANOVA: $F_{2,129} = 1.17$; $P = 0.31$; Fig. 3c) nor did the average mass of sons (ANOVA: $F_{2,113} = 0.59$; $P = 0.55$) or the average mass of daughters (ANOVA: $F_{2,102} = 0.55$; $P = 0.58$). Mass of progeny was also unrelated to maternal mass (ANOVA: $F_{2,128} = 0.01$; $P = 0.94$). The predicted negative association between number of progeny and mass of progeny was weak (ANOVA: $F_{2,124} = 2.23$; $P = 0.14$), and the slope of this relationship did not differ among morphs (ANOVA: $F_{2,124} = 0.32$; $P = 0.73$). Finally, progeny sex ratio was unrelated to maternal morph (GLM: $\chi^2 = 0.17$, d.f. = 2; $N = 134$; $P = 0.92$; Fig. 3d).

Experimentally assessing costs of reproduction

As previously reported (Cox & Calsbeek, 2010b), the elimination of reproduction via OVX resulted in dramatic increases in survival and growth, relative to SHAM females (Table 1; Fig. 4). However, we did not observe any morph differences in overall survival or growth (Table 1). Moreover, we did not detect any significant morph × treatment interactions with respect to survival or growth (Table 1; Fig. 4), indicating that these costs of reproduction are similar across morphs. The only exception to this pattern is that survival of SHAM females was higher than survival of OVX females within the B morph in 2009, whereas survival of OVX was always higher than that of SHAM in every other morph and within the B morph in every other year. Hence, we observed a significant morph × treatment interaction with respect to survival in 2009 (logistic: $\chi^2 = 8.85$, d.f. = 2; $N = 211$; $P = 0.012$). However, this result is only marginally significant when considering each annual replicate as an additional test of the same hypothesis (Dunn–Sidak correction for $k = 3$ multiple tests, $\alpha' = 0.017$). Moreover,

Table 1 Statistical models testing for fixed effects of morph (B, DB, D), surgical treatment (OVX, SHAM) and body size (SVL or mass) on survival (logistic regression) and growth (linear mixed models). All models included year (2007, 2008, 2009) as a random effect. Morph*treatment interactions were used to test the hypothesis that morphs differ in costs of reproduction. Three-way interactions between size, morph and treatment were never significant and were omitted from final models. Bold font denotes significant ($P < 0.05$) effects.

Survival (n = 590)	d.f.	χ^2	P
Morph	2	4.12	0.1273
Treatment	**1**	**16.92**	**< 0.0001**
Morph*treatment	2	0.97	0.6167
Growth in length (n = 195)	**d.f. (N,D)**	**F**	**P**
Morph	2,184	2.18	0.1156
Treatment	**1,183**	**19.21**	**< 0.0001**
Morph*treatment	2,184	0.18	0.8341
SVL	**1,184**	**141.18**	**< 0.0001**
SVL*morph	2,184	2.60	0.0774
SVL*treatment	1,184	3.89	0.0501
Growth in mass (n = 195)	**d.f. (N,D)**	**F**	**P**
Morph	2,183	0.52	0.5951
Treatment	**1,183**	**31.83**	**< 0.0001**
Morph*treatment	2,184	0.10	0.9077
Mass	**1,185**	**48.89**	**< 0.0001**
Mass*morph	2,184	0.70	0.4961
Mass*treatment	**1,183**	**7.16**	**0.0081**

B, bar; D, diamond; DB, diamond–bar; SVL, snout-vent length; OVX, ovariectomize.

this pattern is contrary to that observed in the B morph in two previous years, and it is driven by data from the least common morph in the year with the lowest overall survival (i.e. within the B morph, only 10 of 28 SHAM and four of 27 OVX survived in 2009). We did not observe significant morph × treatment effects on growth in SVL or mass in any individual year of study.

Elimination of reproduction via OVX also resulted in increased immune function (ANOVA: $F_{1,195} = 22.44$; $P < 0.0001$) and haematocrit (ANOVA: $F_{1,106} = 42.87$; $P < 0.0001$) relative to SHAM females (Cox *et al.*, in press). However, we did not detect significant morph × treatment interactions with respect to either immune function (ANOVA: $F_{2,195} = 1.62$; $P = 0.20$; Fig. 4c) or haematocrit (ANOVA: $F_{2,106} = 0.34$; $P = 0.71$; Fig. 4d). Contrary to our predictions, parasitaemia did not differ between OVX and SHAM (ANOVA: $F_{1,146} = 2.66$; $P = 0.11$). Nor did we observe any morph-specific differences in the effect of surgical treatment on parasitaemia (ANOVA: morph × treatment: $F_{2,146} = 0.07$; $P = 0.93$). However, we did detect a marginally significant overall difference in parasitaemia among morphs (ANOVA: $F_{2,146} = 2.85$; $P = 0.061$; Wilcoxon: $\chi^2 = 6.16$, d.f. = 2; $P = 0.046$). Across both SHAM and OVX treatments, D females had slightly higher parasite loads (mean ± 1 SE = 12.90 ± 2.34 parasites per 10^3 red blood cells) than either B (8.48 ± 2.99) or DB females (8.68 ± 1.41). This result would not be significant if we considered our separate response variables (survival, growth in SVL, growth in mass, immune function, haematocrit, parasitaemia) as multiple tests of the same hypothesis that morphs differ in the cost of reproduction (Dunn–Sidak correction for $k = 6$ multiple tests, $\alpha' = 0.0085$).

Natural variation in survival, growth and physiology

Comparisons within the SHAM group of reproductive females did not reveal any natural morph differences in survival (logistic: $\chi^2 = 3.84$, d.f. = 2; $N = 297$; $P = 0.15$), growth in SVL (LMM: $F_{2,71} = 2.62$; $P = 0.08$), growth in mass (LMM: $F_{2,74} = 0.70$; $P = 0.50$), immune function (ANOVA: $F_{2,93} = 0.76$; $P = 0.47$), haematocrit (ANOVA: $F_{2,47} = 0.22$; $P = 0.80$) or parasitaemia (Wilcoxon: $\chi^2 = 3.33$, d.f. = 2; $N = 71$; $P = 0.19$). Patterns of overall survival and growth differed across years, but this interannual variation was consistent across morphs. Within the SHAM group, we did not detect any pairwise correlations between growth, immune function and $\log_{10}$-parasitaemia (all $r^2 < 0.1$; all $P > 0.2$). Moreover, morphs did not differ in the slopes of the relationships between any of these traits (all $P > 0.2$).

Discussion

Although female brown anoles express a genetically based polymorphism that is subject to natural selection (Calsbeek *et al.*, 2008, 2010b), the results of our study strongly suggest that this morph-specific selection is not driven by underlying differences in reproductive strategies. Three years of cross-sectional sampling revealed that the frequency of egg production in the wild is similar among morphs (Fig. 2), and two generations of captive breeding data revealed no differences in the number, frequency, body mass or sex ratio of progeny produced by each morph (Fig. 3). Moreover, reproductive females of each morph did not differ in their survival, growth, immune function, haematocrit or parasitaemia in the wild (Fig. 4). Finally, phenotypic manipulations of reproductive investment confirmed significant costs of reproduction with respect to survival, growth, immune function and haematocrit, but the magnitude of these costs did not differ among morphs (Fig. 4).

Despite considering multiple aspects of reproductive investment and its associated costs, we found only two patterns that suggested potential differences among morphs. First, whereas OVX consistently survived better than SHAM in each year of study for D and DB morphs, SHAM survived better than OVX in 2009 for B morphs. In other species, polymorphisms are maintained by temporal variation in environmental factors, which alters

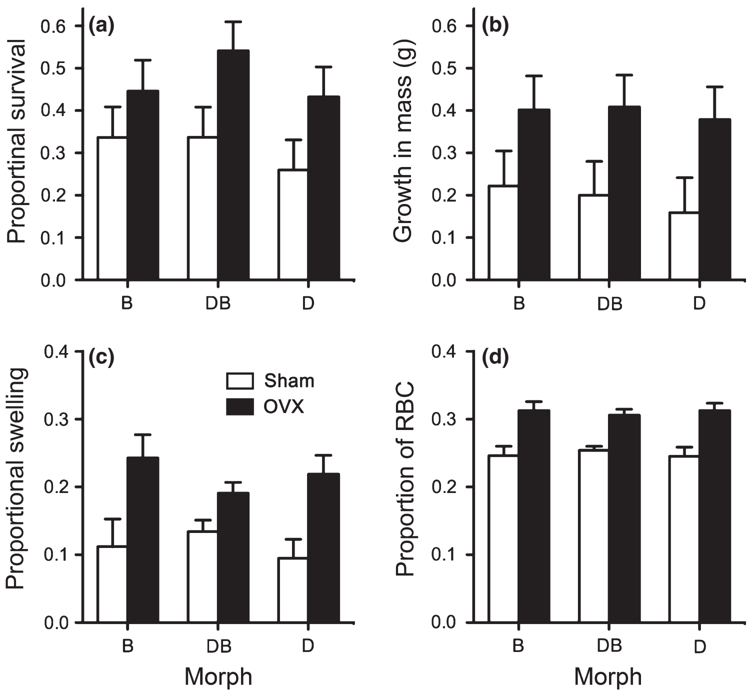

Fig. 4 Effects of morph and surgical treatment on (a) survival, (b) growth, (c) immune function (swelling response to phytohemagglutinin) and (d) haematocrit (proportion of red blood cells to total blood). Data in a are least-square means (± 1 SE) from models accounting for random effects of year. Data in b are least-square means (± 1 SE) from models accounting for initial body mass and random effects of year. Data in c and d are means (± 1 SE).

the relative fitness payoffs associated with alternative strategies (Sinervo *et al.*, 2000; Sinervo, 2001; Vercken *et al.*, 2010). Although it is possible that the interactions we observed could reflect analogous temporal variation in morph-specific reproductive investment, it is difficult to reconcile these complex patterns with any fundamental difference in underlying reproductive strategies. Moreover, after accounting for this temporal variation, we did not find any evidence that reproductive trade-offs differed among morphs. The second morph difference that we observed was a marginally higher level of parasitaemia in D relative to DB and B morphs, similar to morph differences in parasitaemia reported for other polymorphic lizards (Calsbeek *et al.*, 2010a). Although differences in parasite resistance could contribute to alternative fitness optima in each morph (Calsbeek *et al.*, 2010a), our experimental results indicate that levels of parasitaemia are not directly related to reproductive investment (Cox *et al.*, in press). Moreover, the weak statistical effect of morph on parasitaemia was not significant after adjusting Type I error rates for multiple testing. Thus, we conclude that the genetically based dorsal pattern polymorphism found in brown anoles does not correspond to alternative female reproductive strategies *per se*.

The absence of morph-specific reproductive strategies in female brown anoles stands in contrast to patterns observed in two other polymorphic lizards, the side-blotched lizard (*Uta stansburiana*) and the common lizard (*Zootoca* [*Lacerta*] *vivipara*). One potential explanation for this discrepancy may be that we have not yet examined the appropriate combinations of physiological, morphological or behavioural axes along which female morphs differ. Alternative reproductive strategies of these other polymorphic species often manifest as reaction norms or interactions that are most evident when assessed in the light of relevant physiological, ontogenetic or environmental variation (Svensson *et al.*, 2001, 2002; Lancaster *et al.*, 2007; Vercken *et al.*, 2007). Indeed, many of the morph-specific patterns of survival that we have previously documented in brown anoles occur in the form of correlational selection on trait combinations and depend critically upon local population density (Calsbeek *et al.*, 2008, 2010b). Although complex patterns of phenotypic integration and context dependence are expected for alternative reproductive strategies (Sinervo & Calsbeek, 2003; Vercken *et al.*, 2010), other polymorphic systems also exhibit more straightforward differences in basic aspects of reproduction such as clutch size, egg size or egg viability (Sinervo *et al.*, 2000; Vercken *et al.*, 2007). By contrast, we did not detect any morph-specific differences in fundamental reproductive traits such as the number, frequency, size or sex ratio of progeny.

Correspondence between colour polymorphism and alternative reproductive tactics often occurs specifically because colouration functions as a visual signal that mediates social interactions among morphs (Sinervo & Lively, 1996; Sinervo, 2001; Vercken & Clobert, 2008). We do not presently have any evidence that dorsal pattern acts as a social signal in brown anoles, which may be another reason why our results differ from those found in other species. However, our hypothesis that morphs correspond to alternative reproductive strategies was based on three lines of preliminary evidence linking the survival of female morphs to variation in immune function and density (Calsbeek *et al.*, 2008, 2010b), linking immune function and survival to reproductive investment (Cox & Calsbeek, 2010b; Cox *et al.*, in press) and linking geographical variation in morph frequency to variation in life history (Calsbeek *et al.*, 2010b; Cox & Calsbeek, 2010a). We emphasize that our evidence for correlated geographical variation in morph frequency, survivorship and reproductive effort is based on a comparison of only two islands. Further sampling across the Bahamas archipelago would help to clarify geographical patterns in morph frequencies and determine whether this polymorphism is correlated with life-history variation. However, the absence of any correspondence between dorsal pattern and reproductive investment in the present study strongly refutes this hypothesis. This raises the question of what selective forces, if any, are acting to maintain this polymorphism.

One process that is fundamental to the maintenance of polymorphism is negative frequency-dependent selection, or rare morph advantage, which can arise from a variety of underlying dynamics (Sinervo & Lively, 1996; Sinervo, 2001; Svensson *et al.*, 2005; Gray & McKinnon, 2006; Sinervo *et al.*, 2007). By contrast, the only pattern of frequency dependence for which we have evidence in anoles is an apparent increase in the relative fitness of DB females as their frequency increased across temporal and spatial replicates (Calsbeek *et al.*, 2010b). The mechanisms that could create this positive frequency dependence are unclear, but in no instance we have observed the contrasting pattern of negative frequency dependence, which is thought to be more relevant to the maintenance of polymorphism (Gray & McKinnon, 2006; Olendorf *et al.*, 2006).

A second possibility is that the high fitness of DB females, coupled with the reinforcing effect of positive frequency dependence (Calsbeek *et al.*, 2010b), could maintain polymorphism by a process analogous to over-dominance (i.e. heterosis or heterozygote advantage; Gray & McKinnon, 2006). Although DB females are phenotypically intermediate to D and B, breeding studies indicate that the polymorphism is controlled by at least two loci (Calsbeek *et al.*, 2010b). This suggests a process more complex than over-dominance at a single locus. Nonetheless, several two-locus models provide hypothetical mechanisms by which DB phenotypes can be produced from genotypes containing D and B alleles, thus potentially maintaining genetic variation despite selection favouring DB phenotypes (Calsbeek *et al.*, 2010b). Our understanding of the exact genetic mechanisms that produce this dorsal pattern polymorphism is complicated by the fact that males do not express the polymorphism, thus their genetic contributions to progeny are difficult to quantify. However, given the historical interest in over-dominance as a mechanism for the maintenance of colour polymorphism (Gray & McKinnon, 2006), this possibility deserves further attention.

Many colour and pattern polymorphisms also reflect environmental variation in selection for predator avoidance via crypsis (Nachman *et al.*, 2003; Nosil, 2004; Rosenblum, 2006), mimicry (Kaplan, 2001; Harper & Pfennig, 2008) or correlated escape behaviours (Brodie, 1992; Lancaster *et al.*, 2010). More generally, the antagonistic effects of sexual selection for conspicuous displays vs. natural selection for crypsis are thought to underlie sex differences in colouration in many organisms (Galeotti *et al.*, 2003). In similar fashion, the sex-limited polymorphism of brown anoles could reflect a combination of sexual selection favouring a highly visible phenotype in males vs. natural selection favouring alternatively cryptic patterns in females. For example, a longitudinal stripe could render B females cryptic when perched on small branches, whereas a diamond pattern could disrupt the image of a D female against a complex background of leaf substrate. However, female morphs do not appear to differ in their antipredator behaviour, and experimental manipulations of the predator guilds on replicate island populations actually suggest that intermediate DB females have the highest relative fitness in the presence of bird and snake predators (R. Calsbeek & R.M. Cox, unpublished data). Thus, we currently lack clear evidence that differences in susceptibility to predators contribute to alternative fitness optima of anole morphs.

In summary, we have shown that the sex-limited dorsal pattern polymorphism exhibited by female brown anoles does not correspond to alternative reproductive tactics, at least with respect to the various aspects of reproductive investment and its associated physiological and fitness costs that we measured in this study. This differs from the pattern observed in several other species of polymorphic lizards and suggests that the polymorphism in brown anoles is either nonadaptive or maintained by selective pressures unrelated to reproduction and life history. Although our phenotypic manipulations clearly refute the hypothesis that polymorphism corresponds to alternative reproductive tactics in brown anoles, we emphasize that similar experimental approaches should nonetheless prove informative in assessing variation in reproductive trade-offs within and among other species.

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