Differential fitness effects of immunocompetence and neighbourhood density in alternative female lizard morphs

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Summary

1. A growing number of studies demonstrate that natural selection acts on traits important in whole animal performance and physiology.

2. Here we describe a heritable polymorphism in female dorsal pattern in the lizard *Anolis sagrei* (Dumeril & Bibron 1837).

3. Morphs did not differ in body size or habitat use (perch diameter), however, we show that the social environment, estimated by the number of female neighbours, had different selective effects on alternative morphs in nature.

4. We show that morphs displayed a significantly different immune response to phytohaemagglutinin. Furthermore, natural selection differentially acted on combinations of female morph and immunocompetence, favouring high levels of immune function in one morph and low levels of immune function in the other.

5. We discuss the possibility that morph-specific investments in life-history traits may lead to correlational selection between traits, even when those traits are likely to be determined by different genetic loci.

Key-words: correlational selection, immunocompetence, islands, lizard, natural selection, phenotypic polymorphism.

Introduction

Traditionally, studies of natural selection have focused on variation in one or a few traits, and how this variation affects fitness (Kingsolver *et al*. 2001). More recently, however, there has been interest in understanding how selection acts on higher-level or whole animal performance measures such as running performance and immune function (Husak *et al*. 2006). Performance may be considered a higher level of organization because physiological traits such as stamina and immune function, etc., result from the culmination of many underlying physiological processes. Arnold (1983) proposed that demonstrating adaptation in nature requires linking variation in morphology to performance, and variation in performance to fitness. While much progress has been made linking morphological traits with performance (Kingsolver & Huey 2003), considerably less is known about how performance

and fitness are directly related, especially in natural populations.

One performance trait that may be related to fitness is the ability to mount an effective immune response, because a good immune defence mechanism may be crucial in preventing parasite invasions (Janeway, Travers & Walport 2001). Variation in pathogen resistance is likely to contribute to differences in survival (Saino, Bolzern & Moller 1997) and reproductive success (Hamilton & Zuk 1982; Saino *et al*. 1997). Consequently, a growing number of studies have demonstrated selection on immune function in a variety of taxa including birds (Saino *et al*. 1997), lizards (Svensson, Sinervo & Comendant 2001) and insects (Rolff & Siva-Jothy 2004). However, investing in immune function may be costly (Svensson *et al*. 2001; Bonneaud *et al*. 2003). Increased investment in immune function can lead to trade-offs with other traits that are important for fitness, including parental care (Moreno *et al*. 2001), mating behaviour (McKean & Nunney 2001) and sexual ornamentation (Verhulst, Dieleman & Parmentier 1999). Thus, the optimal investment in immunity will depend not only on the risk of infection but also on the competing needs of other life-history traits.

Differential investment in life-history traits may lead to correlational selection favouring alternative life-history

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strategies with different fitness optima. This is thought to play a key role in the evolution of polymorphism (Sinervo & Calsbeek 2003). Correlational selection may build genetic correlations within individuals that express different forms of the polymorphism (hereafter 'morphs') and may therefore play a crucial part in the maintenance of genetically based polymorphisms. For example, Svensson *et al.* (2001) detected correlational selection on female morph and immune response in the side-blotched lizard *Uta stansburiana*, in which yellow and orange females were selected for high and low immune function, respectively. These effects were mediated by social crowding (i.e. neighbourhood density), which was found to have a higher immunosuppressive effect in orange females, suggesting that the costs of immunity were morph-specific.

Here, we describe a sex-limited polymorphism in which female *Anolis sagrei* lizards exhibit one of two discrete dorsal patterns or a third intermediate pattern. The first of the discrete morphs is characterized by two longitudinal-stripes running the length of the back. The second morph exhibits a series of interconnected diamonds also running the length of the dorsal midline. The intermediate morph is variable, and exhibits a pattern ranging from rounded diamonds to irregular stripes (Fig. 1). For simplicity, we hereafter refer to the striped morph as 'bar', the diamond morph as 'diamond' and the intermediates as 'diamond–bar' females. A dorsal pattern polymorphism has been previously described in *A. sagrei* by Schoener & Schoener (1976) in a study from the Little Bahama Bank. While the striped female morph studied by Schoener & Schoener (1976) is similar to the bar females described here, the alternative diamond morph in our study apparently did not exist on the Little Bahama Bank, at least not at the time of Schoener & Schoener's (1976) study.

We present results from a field study of insular *A. sagrei* females, in which we explored whether female morphs differed in a fitness-related life-history trait (immunocompetence) in two study populations, and subsequently estimated morph-specific natural selection on this trait. When selection acts on alternative phenotypes and is related to population density, then the fitness of individuals should depend on the phenotypes of surrounding individuals (Sinervo, Bleay & Adamopoulou 2001). More specifically, and based on the results of Svensson *et al.* (2001) on side-blotched lizards described above, we tested whether: (1) morphs were heritable; (2) morphs differed in immunocompetence; (3) morphs were differentially affected by neighbourhood density; (4) social crowding differentially affected immune response between the morphs; and (5) natural selection favoured differential investment in immune function between the morphs.
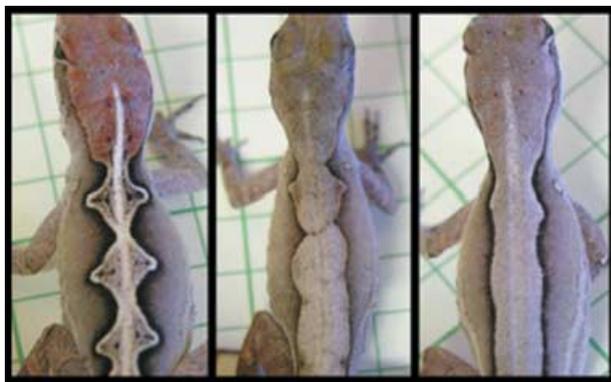
Methods

STUDY SITES AND SYSTEM

The brown anole, *Anolis sagrei*, is a small (< 70 mm snout–vent length; SVL) semiarboreal lizard widely distributed throughout tropical and subtropical areas. In our study populations in the Bahamas, males and females both defend small home ranges that often overlap with the home ranges of several other individuals. *Anolis sagrei* in our study population usually begin breeding in late March. Females lay one or two eggs at approximately 10-day intervals throughout the breeding season, which typically ends by September Most individuals (> 85%) in our study populations mature and die in a single year and the bulk of mortality occurs during the summer months (May–October; Calsbeek & Smith 2007).

This study took place from May–October during 2005 on two small islands near Georgetown, Great Exuma, Bahamas. We studied a natural population of lizards on Kidd Cay (c. 1500 m²), and a second population on Flamingo Bay Cay (c. 750 m²) that we introduced from an adjacent (< 2 km away) study site during May 2005 as part of a long-term experimental study of natural selection (Calsbeek & Smith 2007). The two islands have similar vegetation, dominated by palms *Pseudophoenix* spp., Australian pines *Casuarina equisetifolia*, sea-grape *Coccoloba uvifera*, sea hibiscus *Hibiscus tiliaceus* and buttonwood *Conocarpus erectus*. The lizards on the two islands are also exposed to similar avian predators (particularly mocking birds *Mimus polyglottos* and herons *Butorides striatus*) but Kidd Cay also supports a terrestrial lizard that may occasionally eat *A. sagrei* (the whiptail lizard *Ameiva*) and that is not present on Flamingo Bay Cay. We captured all lizards on each island by hand or using a silk noose. At capture, females were scored unambiguously as either bar (B), diamond (D), or diamond–bar (DB) based on the presence of longitudinal stripes, diamonds or imperfect polygons, respectively, expressed on the dorsum (Fig. 1). Scoring of the morphs was unambiguous in that both trained and naïve observers classify the females with near perfect agreement (unpublished data). We studied a total of 125 females on Kidd Cay (15 bar, 77 diamond and 33 diamond–bar) and 93 females on Flamingo Bay Cay (nine bar, 58 diamond and 26 diamond–bar).

All females were marked during May and June with unique combinations of coloured elastomer-dye that was injected into the ventral side of the hind and forelimbs (Nauwelaerts, Coeck & Aerts 2000). Tags serve as permanent identification in the wild, allowing us to track the fate of every individual over the course of the study (Calsbeek & Marnocha 2006).

We recorded home range data for females by walking multiple daily transects over the entire Kidd Cay study site for a period of 2 weeks. Individual locations for each lizard were mapped using

Fig. 1. The three alternative phenotypes expressed by female *A. sagrei* on the Great Bahama Bank, from left to right are: diamond, diamond–bar, and bar females. Dorsal pattern morphs are only observed in females. The red head exhibited by the left-most female may be expressed by any of the morphs.

compass bearings and distance measurements from known landmarks or relative to the initial site of capture. We constructed home range maps using the software package MacTurf (DeNardo & Sinervo 1994) and defined neighbours as lizards with coincident or overlapping home range boundaries. The number of female neighbours for each female was used to estimate social crowding (i.e. neighbourhood density; see Svensson *et al*. 2001) because territorial aggression is largely intrasexual in this population. Neighbourhood sizes were only estimated on Kidd Cay.

MEASURING IMMUNOCOMPETENCE

We estimated immunocompetence by injecting females with a novel mitogen (phytohaemagglutinin, PHA-P; Sigma). PHA is a standard estimate of *in vivo* cell-mediated immune response that can be readily performed on animals in the wild (Svensson *et al*. 2001). PHA influences a variety of vertebrate cell types (Bonforte *et al*. 1972; Goto *et al*. 1978; Elgert 1996). Response to PHA is therefore complex (Kennedy & Nager 2006; Martin *et al*. 2006) but nevertheless indicates immune activity and is used as a surrogate for immunocompetence. We stress that we do not discuss here whether a higher swelling is more or less adaptive. Likewise, we do not discuss the advantages or costs of investing in immunity. Rather, we focus uniquely on how female morphs differ in immune swelling.

We challenged females with 0·10 mg PHA dissolved in 0·01 mL phosphate-buffered saline (PBS), injected subcutaneously into the left hind-foot pad. As a control, we injected the same volume of PBS in the right hind-foot pad. Females were held overnight in separate plastic containers. We measured the thickness of each foot pad with dial-callipers (± 0.01 mm) at the injection site, prior to and 24 h post-injection. We assessed the intensity of the immune response as the difference in swelling between the PHA-injected and the control foot pads. We measured immunocompetence for 68 females on Kidd Cay (11 bar, 47 diamond and 10 diamond–bar) and 47 females on Flamingo Bay Cay (eight bar, 20 diamond and 19 diamond–bar).

SELECTION ANALYSES

We assessed survival over the 4-month period from May to October, which includes that time frame during which lizards reach sexual maturity, and also spans nearly the entire breeding season. All surviving lizards on Flamingo Bay and Kidd Cays were recaptured in mid-October to assess selection. As most (> 85%) *A. sagrei* in our study populations mature and die in a single year (Schoener & Schoener 1982; Calsbeek & Smith 2007), the length of our selection study represents the time to reach the end of the breeding season. Thus, our selection study should account for variation in both viability and potential reproductive success. Censuses can be considered exhaustive as they continued for 2–3 weeks, or until three consecutive days of searching turned up no new marked lizards. Lizards not recovered during this period were considered dead; a reasonable assumption as emigration off of study islands is rare, except during major storms (Calsbeek & Smith 2003), none of which affected this study. Recapture efficiencies were *c*. 98% based on a regression of the number of lizards captured each day (log transformed) against cumulative days of capture effort.

We used general linear models to extract selection gradients (Lande & Arnold 1983) for linear (β) and nonlinear (γ) forms of selection, and to test for overdispersion of data. Linear and nonlinear gradients were computed in separate models to minimize the effects of multicolinearity (Brodie, Moore & Janzen 1995).

Assumptions of parametric statistics, especially the assumption of normally distributed residuals, are likely violated by survival estimates in which the dependent variable is binomially distributed (e.g. live/die). We therefore verified significance values for selection gradients using generalized linear models with a logit link function that accounts for binomial error variance. We assigned morphs a numeric dummy variable (0 for bar, 1 for diamond–bar, and 2 for diamond), which allowed us to incorporate an ordinal and stand-ardized value for morph in our statistical tests. All variables used in selection analyses were standardized to mean 0 with unit variance, except survival, which was scaled by the mean survival of all females in the population (Lande & Arnold 1983). Prior to computing selection gradients, we performed a mixed model analysis of variance, with 'study island' as a random factor, to test whether patterns of selection differed significantly between sites. They did not, and data sets were subsequently pooled where appropriate (see Results). We included the covariate SVL to control for variation due to body size in our analyses. All statistical tests were two-tailed.

We visualized fitness surfaces the multivariate cubic-spline (Schluter & Nychka 1994). The cubic-spline is a nonparametric approach to finding the best-fit three-dimensional surface that describes the interaction of multiple traits as they relate to fitness. This method makes no a priori assumptions about shape of the surface, and may be preferable to the standard quadratic surface if parametric esti-mates become biased by the distribution of data (Phillips & Arnold 1989; Schluter & Nychka 1994). We plotted the nonparametric surface for the entire data set (both sites pooled). Data sets from individual sites were not sufficiently large to make use of nonpara-metric surfaces. We therefore report the quadratic surfaces for each individual study plot. The complexity of the pooled surface was low enough that parametric surfaces still provide a useful illustration of the patterns of selection within plots (Phillips & Arnold 1989; Schluter & Nychka 1994; Brodie *et al*. 1995; Blows, Brooks & Kraft 2003; McGlothlin *et al*. 2005).

HERITABILITY OF THE MORPHS

During 2004 and 2005 we initiated a breeding study at the University of California, which was designed to estimate the heritability of a female morph. Males do not express the dorsal polymorphism, and we bred each female to multiple males to maximize the production of half-sibs and control for potential unmeasured sire effects. Each female was housed in a separate 10-gallon terrarium and was provided with full spectrum lighting, and *ad libitum* food (*Achaeta* crickets) and water. Because females were housed individually, the maternity of all offspring was known with certainty. To minimize potential environmental influences on phenotypic variance, all newly hatched progeny were randomized by sib-ship into separate terraria until adulthood. We obtained a total of 65 daughters from 34 different dams in our breeding study.

Progeny morph was scored at hatching by two independent observers (RC and CB). Discrete polymorphisms have been repeatedly shown to be under simple genetic control (Cordero 1990; Smith 1993; Shuster & Sassaman 1996; Sinervo *et al*. 2001) and we hypothesized that the morphs in this system would likewise have a simple mode of inheritance. To test inheritance patterns against a null Mendelian model, we assigned each morph a numeric score based on the presumed number of morph-specific alleles it carried (Sinervo *et al*. 2001). We arbitrarily chose diamond alleles as a baseline for this analysis and assigned bar females '0' (having presumably 0 diamond alleles, or alternatively, 2 bar alleles), diamond bar females '1', and diamond females '2'. We estimated

heritability as twice the slope of the least squares regression of progeny morph on dam morph (Falconer & MacKay 1996). The least squares slope is not biased by ordinal data, however, significance values for this relationship may be biased (Sokal & Rohlf 1995). We therefore estimated the significance of this relationship by performing a weighted mother–daughter ordinal logistic regression using mean allele numbers in progeny as the dependent variable, numbers of dam alleles as the independent variable, and the numbers of progeny in each family as weights.

Results

Heritability was estimated from the slope of the linear regression of daughters morph on dam morph (dummy variables), but we computed the significance of this relationship using weighted ordinal logistic regression. The heritability of female morph was highly significant ($h^2 = 0.78 \pm 0.13$, $\chi^2 = 11.47$, $n = 34$, $P < 0.001$).

Morphs did not differ significantly in SVL in the field (i.e. body size) (ANOVA $F_{2,147} = 1.42$, $P = 0.24$; random factor for island $P = 0.54$) or in the laboratory (ANOVA $F_{1,78} = 0.36$, $P = 0.69$). Neither did they differ in their use of the habitat (e.g. perching diameter ANOVA $F_{2,165} = 0.17$; $P = 0.84$). Response to PHA was positively correlated with body size ($r^2 = 0.05$, $n = 114$, $P = 0.016$), and morphs differed significantly in their response. Bar morphs had the strongest response, diamonds had a weaker response and diamond–bar morphs had the weakest response (immunocompetence based on residual swelling corrected for body size: $\bar{X}_B = 0.42 \pm 0.26$; $\bar{X}_D = 0.10 \pm 0.12$; $\bar{X}_{DB} = -0.37 \pm 0.17$; ANOVA $F_{2,110} = 3.84$, $P = 0.02$; Fig. 2A). In *post-hoc* tests, bar and diamond females both remained significantly different from diamond–bar females.

Female morphs did not differ in the number of neighbours they were surrounded by in the wild ($\bar{X}_B = 7.20 \pm 3.02$; $\bar{X}_D = 9.32 \pm 1.22$; $\bar{X}_{DB} = 6.41 \pm 1.83$; ANOVA $F_{2,97} = 0.95$, $P = 0.39$). Immunocompetence was not correlated with the degree of social crowding ($F_{1,48} = 0.13$, $P = 0.72$; Fig. 2B). Nor did crowding by neighbours have different effects on the immunocompetence of the morphs (morph × density, $F_{2,46} = 1.83$, $P = 0.17$).

During October of 2005, we recaptured 35 of the 125 females (27%) we had marked on Kidd Cay, and 14 of the 93 females (15%) from Flamingo Bay Cay (33 diamond, seven bar, and nine diamond–bar in total). Survival of the female morphs differed significantly as a function of social crowding. We detected a significant interaction between female morph and social crowding on survival on Kidd Cay, the island for which we had collected neighbourhood data. Bar females survived better at low neighbourhood density while diamond and diamond–bar females survived better at higher density (morph × $N_{neighbours}$: $F_{2,93} = 3.29$, $P = 0.04$). Tests of overdispersion of data distributions were not significant in any analyses (all $\chi^2 > 0.05$) and are not reported hereafter.

Across all females, we detected significant directional (i.e. linear) selection favouring increased immunocompetence ($\beta = 0.48 \pm 0.22$, $P < 0.02$; covariate for SVL $P = 0.48$). However, we also detected a significant difference among
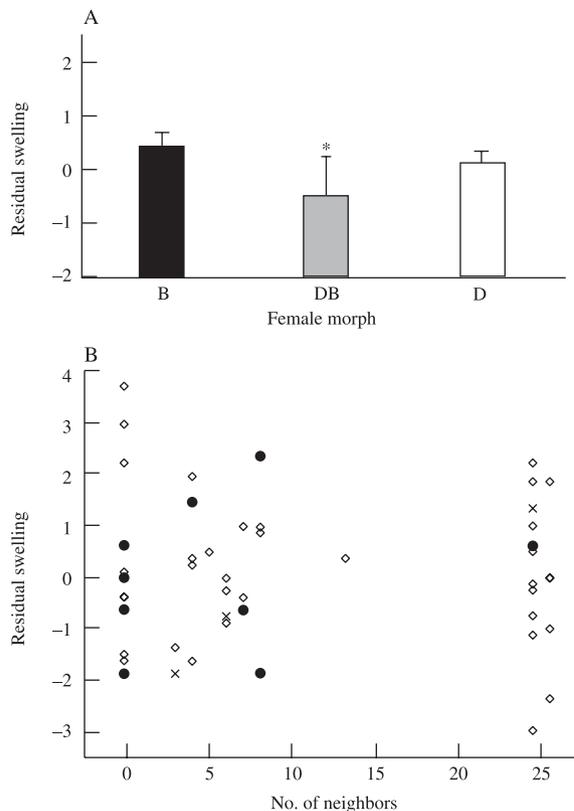
Fig. 2. (A) Morphs showed significant differences in immunocompetence (corrected for body size). Differences remained significant in *post-hoc* tests between diamond–bar females with both bar and diamond females (*). (B) There was no significant relationship between immunocompetence (residuals correcting for variation due to body size) and the degree of social crowding (i.e. density). Symbols show data separately by morph (× = bar, ◇ = diamond, ● = DB).

morphs in this effect. We measured nonlinear correlational selection between female morph and immunocompetence on both plots (pooled $\gamma_{1,2} = 0.55 \pm 19$, $P = 0.004$; see Table 1 for results separately by study island). There was a significant effect of the random factor 'study island' ($P = 0.004$), but no higher-order interaction terms were significant. Thus, survival of morphs differed as a function of immunocompetence in the same way on both islands (Table 1, Fig. 3). Diamond morphs tended to survive well when they exhibited a strong immune response (i.e. large swelling in response to PHA), while bar females tended to survive well when they exhibited a weaker immune response (i.e. little swelling).

Discussion

In sum, we have provided preliminary evidence for the heritable basis of a polymorphism in female dorsal pattern. Female morphs differed in their immune response to PHA. However, morphs did not differ in the number of neighbours they were surrounded by in nature, nor was the response to PHA affected by social crowding. Finally, social crowding had significant impacts on survival to the end of the breeding season, and natural selection appears to have favoured differential investment in immune function among the morphs.

Table 1. Selection gradients for linear (β) and nonlinear selection (γ) showing that correlational selection on both study islands (separately and pooled) favoured bar females with lower swelling (i.e. immunocompetence) and diamond females with higher swelling. Labels are for standardized immunocompetence (Swelling). Morph was converted to a numeric dummy variable (0, 1, 2) to allow standardization

Source for W	d.f.	β/γ	SE	t	P
(A) Kidd Cay					
Swelling	1	0·85	0·35	2·40	0·02
Morph	1	0·19	0·29	0·66	0·51
Swelling^2	1	0·09	0·34	0·28	0·77
Morph^2	1	0·44	0·36	1·23	0·22
Swelling × morph	1	0·99	0·38	2·61	0·01
Residual	41				
(B) Flamingo Bay Cay					
Swelling	1	0·43	0·18	2·37	0·02
Morph	1	0·09	0·19	0·51	0·61
Swelling^2	1	0·16	0·15	1·02	0·31
Morph^2	1	0·09	0·23	0·40	0·69
Swelling × morph	1	0·39	0·23	1·70	0·09
Residual	47				
(C) Both islands pooled					
Swelling	1	0·52	0·18	6·14	< 0·0001
Morph	1	0·25	0·17	1·46	0·15
Swelling^2	1	−0·08	0·14	−0·58	0·56
Morph^2	1	0·36	0·21	1·67	0·09
Swelling × morph	1	0·55	0·19	2·90	0·004
Residual	94				

W, Survival

Though female morphs did not differ in body size or the number of neighbours they were surrounded by in nature, survival of the female morphs differed significantly as a function of social crowding. As such, we have demonstrated significant correlational selection between crowding and morphotype. Results were similar in the natural and introduced populations suggesting that the introduction to a novel habitat did not bias our results (e.g. through costs of dispersal). Correlational selection favours successful trait combinations within individuals (Brodie 1992; Forsman & Appelqvist 1998; Medel 2001; Sinervo *et al.* 2001; Agrawal, Conner & Stinchcombe 2004; McGlothlin *et al.* 2005; Bentsen *et al.* 2006), in this case favouring diamond females with a high immune response to PHA (i.e. immunocompetence) and bar females with a lower immune response (Fig. 3). This result, in contrast to the study by Svensson *et al.* (2001), arose despite the fact that in our study, neighbourhood density *per se* did not affect immunocompetence. We cannot rule out, however, the possibility that the correlational selection reported here may have arisen, at least partly, due to morph-specific costs of immune response mediated by neighbourhood density (i.e. an unmeasured indirect effect).

Correlational selection acting on heritable variation should build genetic correlations over time (Brodie 1989; McGlothlin *et al.* 2005), even if trait loci are not physically linked. We have provided heritability data that are consistent with a genetic basis of dorsal patterning in these lizards. One caveat to consider is that we have not yet demonstrated a genetic mechanism for the inheritance of the polymorphism, and therefore cannot entirely rule out the possibility that some other mechanism (e.g. a heritable physiological difference among females) plays a role in generating the polymorphism. However, preliminary data from our laboratory in which we manipulated food availability and temperature regimes did not affect morph expression or inheritance (E. Marnocha, R. Calsbeek, C. Bonneaud, T.B. Smith, unpublished). Still, without a complete breeding design, we cannot yet definitively conclude that intermediate morphs are not simply members of one of the other two discrete types. Heritability of immune function, while not measured here, has been demonstrated in other groups (Svensson *et al.* 2001; Kilpimaa *et al.* 2005). If immunocompetence is also heritable
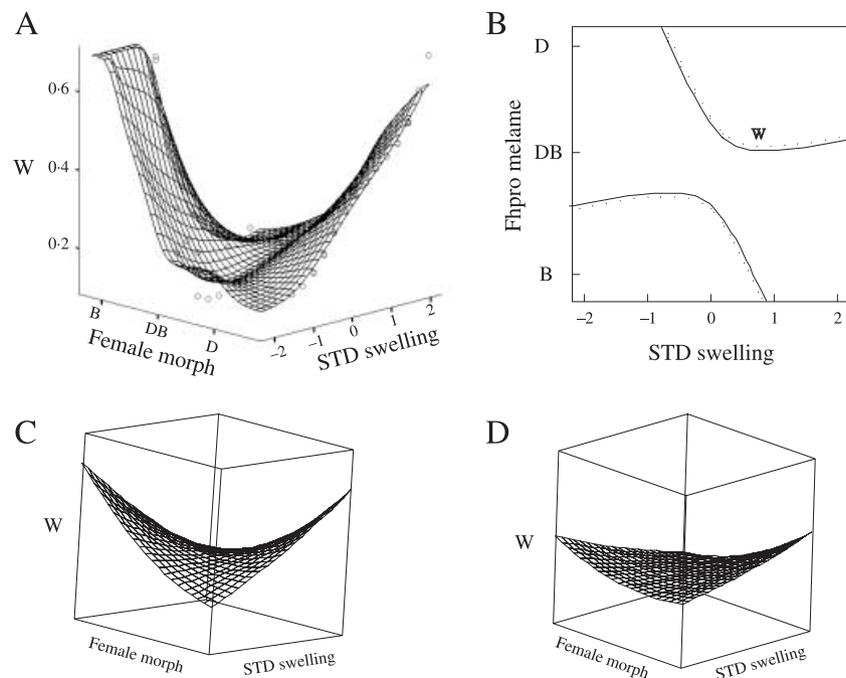
Fig. 3. Fitness surfaces and contour plot for two selection replicates showing correlational selection on female morph and immunocompetence. (A) Shows the fitness surface for both study islands combined and was calculated using projection pursuit regression (cubic-spline regression). (B) Shows the corresponding contour plot. (C,D) These illustrate parametric surfaces for Kidd Cay and Flamingo Bay Cay, respectively. Because of low sample size, cubic spline regression was impractical for surfaces from individual study islands.

in anoles, then the correlational selection described here could result in strong morph-specific linkage disequilibrium between these traits. The maintenance of linkage disequilibrium would then depend on the strength of selection relative to recombination (Lynch & Walsh 1998).

Immune responses can be costly and may influence investment in other life-history traits (Verhulst *et al.* 1999; McKean & Nunney 2001; Moreno *et al.* 2001; Bonneaud *et al.* 2003). Our results indicate that morphs invest differently in immunocompetence, possibly reflecting differences in their life-history strategies. Bar females may invest less in immune function (and/or more in other life-history traits) compared with diamond females. As our results also suggest that social crowding imposes a stronger negative effect on bar females, which subsequently survive poorly at high neighbourhood density, we can hypothesize that the costs of immune activity may differ between the morphs at high density. Indeed, differential investment in immunity might be exacerbated between the morphs at high density. If, for example, bar females experience costs due to increased investment in other life-history traits such as egg mass, then social crowding might not have direct effects on immunocompetence, but could act indirectly through other costs that are associated with density. If investment in these other traits were traded-off with investment in immune function, this could have indirect effects on survival. Future studies aimed at experimentally manipulating life-history costs (Sinervo & Licht 1991) and neighbourhood density could resolve the relative importance of these two potential explanations.

In addition to the correlational selection demonstrated here, morphs could also experience differential natural selection through differences in predation rates (see Brodie 1989). Thus, apostatic selection could have important fitness consequences for the morphs if alternative bar and diamond patterns were differentially visible to predators. Schoener & Schoener (1976) proposed apostatic selection as a possible explanation for why bar females in their study were consistently smaller than the other female morphs. They reasoned that body size and perching habitat are correlated, and that smaller lizards should be found more often on narrow diameter vegetation (Irschick 2000). As longitudinal stripes should confer a crypsis benefit on twigs and other narrow diameter perches, bar females would have a fitness advantage in scrubby habitats. Schoener & Schoener (1976) used the same rationale to explain why the larger body-size males would never express the bar phenotype. Morphs in our study population did not differ in their use of the habitat. However, Stamps & Gon (1983) have shown that avian predators differentially attack alternative dorsal pattern morphs in reptiles and other species, lending further support to the possible role of apostatic selection in *Anolis* females. Future studies will assess the possible importance of both bird and lizard predators acting on *A. sagrei*.

Our results add to the growing body of studies aimed at measuring selection on higher-order traits including behaviour and performance (Husak *et al.* 2006). Though correlational forms of selection are less frequently measured in nature compared with other linear and nonlinear measures (Blows 2007), the parallels between results reported here and in Svensson *et al.* (2001) suggest that correlational selection acting on alternative morphs may be more common in nature than previously thought.

Acknowledgements

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