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Mechanistic and experimental analysis of condition and reproduction in a polymorphic lizard

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Mechanistic and experimental analysis of condition and reproduction in a polymorphic lizard

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Abstract

The importance of genetic and environmental variation in condition in shaping evolutionary trade-offs have recently been subject to much theoretical discussion, but is very difficult to investigate empirically in most field-based systems. We present the results from mechanistic experimental manipulations of reproductive investment and condition in two female colour morphs ('orange' and 'yellow') of side-blotched lizards (*Uta stansburiana*). We investigated the interactions between throat colour morphs, condition, local social environment and female survival using path-analysis. Using follice-ablation experiments, we show that large clutch size has a negative effect on field survival among yellow females, and that this effect is partly mediated by immunosuppressive effects of large clutches. In orange females these effects were less pronounced, and there was a negative survival effect of strong antibody responses. Hence, we experimentally confirmed our previous findings of correlational selection between female morphotype and immunocompetence, an important condition trait. Manipulation of corticosterone revealed multiple ('pleiotropic') direct and indirect effects of this hormone on both condition and reproductive traits. We argue that interaction effects (e.g. between local environments and genotypes) could explain a substantial fraction of variation in condition and reproduction in natural populations. Increased attention to such interaction effects and their fitness consequences will provide novel insights in field studies of selection and reproductive allocation.

Introduction

Evolutionists have recently directed their attention to the physiological mechanisms and developmental basis of important phenotypic traits and fitness components (Nijhout & Emlen, 1998; Schlichting & Pigluicci, 1998). Although traditional quantitative genetic approaches and ecological analyses of fitness variation continue to be important, workers are now also focusing much on the relationship between physiological (or functional) and genetic architecture (Zera *et al.*, 1998; Ketterson & Nolan, 1999; Sinervo, 1999).

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Recent models suggest that large variation in condition can mask trade-offs between fitness components (van Noordwijk & de Jong, 1986; Price *et al.*, 1988; Houle, 1991; Rowe & Houle, 1996). It is therefore necessary to measure important condition traits and incorporate such information in field studies of natural selection. Similarly, when individuals in a population reside in environments of different quality, micro-environmental heterogeneity will confound global estimates of selection from a larger population (Brandon, 1990; Wade & Kalisz, 1990). One solution to these problems can be to incorporate measurements of local environments into path-analytical models (Kingsolver & Schemske, 1991; Sinervo & DeNardo, 1996; Svensson *et al.*, 2001a). Of particular interest are local social environments, such as the number of neighbours or phenotype frequency-distributions (e.g. Sinervo & Lively, 1996; Svensson &

Sinervo, 2000; Svensson *et al.*, 2001a). Path analysis is a flexible statistical tool to analyse such situations, because both direct and indirect effects of local environments can be estimated through the method of path coefficients (Kingsolver & Schemske, 1991; Lynch & Walsh, 1998).

Recently, evolutionary ecologists have developed several sophisticated experimental techniques that allow them to manipulate physiological mechanisms (e.g. hormones) behind trade-offs (Ketterson & Nolan, 1999; Sinervo, 1999). Such manipulations give a more realistic picture of trade-offs than traditional manipulations of single traits (Sinervo & Basolo, 1996). Physiological manipulations may also elucidate important issues in evolutionary biology such as pleiotropy (multiple effects of a gene on several traits) and physiological epistasis (interactions between different genes in phenotype formation) (Ketterson & Nolan, 1999). Just like the external environment may affect condition traits in both direct and indirect ways, hormonal manipulations of the internal physiological milieu could affect condition through several different pathways.

In this paper, we merge such experimental physiological techniques with measurements of condition and path-analysis of local social environments. We present data from an experimental study of side-blotched lizards (*Uta stansburiana*), an animal characterized by a heritable and conspicuous throat colour polymorphism (Sinervo & Lively, 1996). Throat colour can be viewed as a 'genetic marker' and is genetically correlated with several disparate behavioural, physiological and reproductive traits (Sinervo *et al.*, 2000a; Svensson *et al.*, 2001a, b). Trait differences between morphs presumably arise from a combination of pleiotropic effects of steroid hormones, epistatic interactions between different loci and correlational selection that refines and shapes morphs (Sinervo & Svensson, 2002). We manipulated clutch size, egg size, condition and survival in the field using a series of previously developed techniques (Sinervo & Licht, 1991; Sinervo & DeNardo, 1996) and studied both direct and indirect effects of these experimental manipulations on two condition traits; postlaying mass and immunocompetence.

The main question in this study concerns how variation in condition is strongly affected by the local environment, by genetic background and by the interaction between the local environment and genotype. Furthermore, if variation in condition has different fitness consequences for different genotypes, it is possible that different optimal character combinations within a population and correlational selection occurs.

Materials and methods

Study organism, field work and general methods

We studied a free-living population of side-blotched lizards, *U. stansburiana*, at Los Banos, Merced County in the inner coast range of California, during 1998 and 1999 (see Sinervo, 1998, 1999 for natural history details). At our main study site, survivors from previous years (mainly hatchlings from our maternal field pedigrees) are recorded and new individuals (immigrants) are marked (toe-clips) for future identification. Captured animals are weighed to the nearest 0.1 g and their snout-vent length (SVL) is measured to the nearest 0.5 mm. Animals receive paintmarks on their backs for future identification in the field during mapping of territories. The mean number of days (±SE) that a female was observed during the reproductive season was 8.68 (±0.46). After biometric measurements and individual markings, all animals are released in the field again at their capture sites. Animals were later re-captured to record reproductive development and subsequently brought into our oviposition laboratory (see below). On average, each female was captured 4.19 (SE = 0.12) times per season. For further details about general field work, see Svensson *et al.* (2001a).

Genetics and natural history of throat colour morphs

As in several other lizard species, males and females in our population occur in discrete and heritable throat colour morphs that also differ in behaviour, life-history traits and physiology, including hormone levels (Sinervo & Lively, 1996; Sinervo *et al.*, 2000a, b; Svensson *et al.*, 2001a, b). When females start to ovulate, the two female throat colour morphs appear: 'orange' and 'yellow'. The onset of throat colouration is governed by circulating hormone levels in adults, including hormonal secretion (progesterone and testosterone) of the growing follicles (Cooper & Greenberg, 1992; Moore *et al.*, 1998; T. Comendant, B. Sinervo, J. Wingfield & E. Svensson, unpublished data). Parent–offspring regressions have revealed that both male ($h^2 = 0.87$–0.96) and female ($h^2 = 0.48$) throat colour morphs are heritable, and maternal effects do not seem to have inflated these high heritabilities (Sinervo & Lively, 1996; Zamudio & Sinervo, 2000; Sinervo *et al.*, 2001). Furthermore, throat colour is genetically correlated with several other behavioural, physiological and reproductive traits (Sinervo *et al.*, 2000a, 2001; Svensson *et al.*, 2001b).

Oviposition and incubation routines

Near-term gravid females that were about to lay were captured and brought into the laboratory to oviposit their eggs. Reproductive state was determined by abdominal palpation every week during the first 14 days of the vitellogenic cycle (~24 days) and every 2–4 days during the last 10 days after ovulation (Sinervo & Licht, 1991). For details about lizard husbandry, laboratory conditions and general routines see Sinervo & Doughty (1996). After females had laid eggs, they were released in the field at their capture sites within 1–3 days. The average

number of days (±SE) between the last field capture prior to oviposition in the laboratory and release in the field again was 15.52 (±0.61) days. During daily visits to the study area, we captured all females that survived to produce second, third and later clutches and we were thereby able to record survival between the completion of the first clutch and production of the second. Mean female survival (±SE's) between the first and second clutch was 0.588 (±0.086) in 1998 and 0.527 (±0.068) in 1999.

Experimental manipulations

During both 1998 and 1999 we performed follicle ablation experiments according to the protocol developed by Sinervo & Licht (1991). Although experimental females produce larger eggs following follicle ablation, this compensatory follicle growth is not complete, resulting in lower total clutch mass compared with control females (Sinervo & Licht, 1991; Sinervo & DeNardo, 1996). We ablated females in the field during late March or early April in 1998 and 1999. Manipulations were performed on average 17.5 (±1.4) days prior to bringing females into the lab to obtain their eggs. In addition, we performed sham-manipulation in some females by only subjecting them to surgery but not ablating the follicles (these females have similar survival as unmanipulated control females; Sinervo & DeNardo, 1996).

We also performed corticosterone manipulations (DeNardo & Sinervo, 1994a, b; Sinervo & DeNardo, 1996) in both 1998 and 1999. Exogenous corticosterone (B-implants) was delivered in silastic implants, and doses were adjusted to increase cortiocosterone in plasma to levels near the upper physiological levels that have been detected in previous field studies of *U. stansburiana* (DeNardo & Sinervo, 1994a,b). Our preliminary data indicate that the average corticosterone-titres increased by approximately 20% in the B-implanted females, compared with controls (least square means: controls: 49.07 ± 2.97 ng mL^{-1}; B-impanted: 60.03 ± 2.97 ng mL^{-1}; T. Comendant, B. Sinervo, J. Wingfield & E. Svensson, unpublished data). Sham-manipulated females were implanted with empty silastic implants soaked in saline. Both sham-manipulations and B-manipulations were performed in late February or early March on average 59.5 (±1.8) days prior to last capture in the field prior to oviposition of the first clutch.

As both our experimental manipulations may affect one or several steroid hormones that are also important in governing the development of throat colouration (Moore *et al.*, 1998), the proportion of orange and yellow females in the different experimental categories could be affected. This would lead to an inbalanced data set and would affect our conclusions. However, the proportion of orange females did not differ significantly between the experimental categories (Controls: 35%; follicle-ablations: 34%; B-implanted: 30%; $\chi^2_{|2|} = 0.52$, n.s.).

Immunization procedures and assessment of antibody responses

In both 1998 and 1999, we performed immunizations of female lizards to measure immunological condition. By stimulating an animal's immune system with a novel antigen (protein) an antibody response is elicited. The strength of this antibody response is routinely used as a measure of humoral immunocompetence ('antibody responsiveness') by workers in veterinary medicine and ecological immunology (see e.g. Cheng *et al.*, 1991; Luster *et al.*, 1993; Svensson *et al.*, 1998). Most vertebrates, including lizards (Saad *et al.*, 1990; Svensson *et al.*, 2001a), can mount antibody responses against a wide diversity of antigens that have previously not been encountered by the individual. Strength of immune responses reflects both genetic and environmental variation due to nutritional state or incidence of diseases (Cheng *et al.*, 1991; Svensson *et al.*, 2001b). A stronger immune response indicates stronger defence against novel infections, and antibody responses are therefore typically and positively correlated with general resistance to diseases and parasites (see Luster *et al.*, 1993). Although no single specific immunocompetence measure can capture all the complexities of the vertebrate immune system, experimental challenge studies like ours are more informative than are correlative approaches based on leucocyte counts or parasite loads (Svensson *et al.*, 1998; Claus Koch, personal communication). Details about antigens, immunization procedures, analyses of antibody responses and ELISA-protocol have been presented elsewhere (see Svensson *et al.*, 2001a).

Sample sizes, statistics and general approach

We incorporate data from unmanipulated females ('controls'; Svensson *et al.*, 2001a) as a benchmark to compare the data from our two experiments. We first investigate the experimental effects on clutch size, egg mass and immunocompetence using data from control females, follicle-ablated and B-implanted females and sham-manipulated females. As the experimental females differed significantly from both the sham- and the control-females in almost all cases, we proceed by more detailed investigations of the experimental effects using data only from the experimental and the control females (excluding the shams). The reasons for this is that for some of the sham-females we did not have a complete data set on the social environment and we also wanted to describe our experimental manipulations using dichotomized dummy variables (control: 0; experiment: 1), rather than a more complicated three-state variable (e.g. Figs 7 & 8). The total, complete data set, in this study was 147 females, consisting of 63 control (unmanipulated) females, 29 follicle-ablated females, 28 corticosterone-implanted females and 29 sham-manipulated females

Table 1 Model of how antibody responsiveness (dependent variable) of female lizards is influenced by the number of orange neighbours, female morphotype (yellow = 0, orange = 1), experimental category (control = 0, follicle-ablation = 1) and all two-way interactions. Sample sizes: 45 control females and 31 follicle-ablated females. Note the statistically significant interaction term between female morphotype and number of orange neighbours; this reflects the different morph-specific slopes of reaction norms towards the social environment (Fig. 3). Type III sums of squares. Full model: $F_{6,69} = 3.010$; $R^2 = 0.207$; $P < 0.05$.

Source	d.f.	MS	F	P
No. orange neighbours	1	821.838	6.579	0.012
Female morph	1	635.750	5.089	0.027
Experimental category	1	511.667	4.096	0.047
Morph × orange neighbours	1	798.312	6.390	0.014
Exp × orange neighbours	1	13.840	0.111	0.740
Exp × morph	1	11.218	0.090	0.765
Residual	69	124.925		

Dependent variable: antibody responsiveness.

Table 2 Model of how postlaying mass (dependent variable) of female lizards is influenced by the number clutch size, female morphotype (yellow = 0, orange = 1), experimental category (control = 0, follicle-ablation = 1) and all two–way interactions. Sample sizes: 43 control females and 28 follicle-ablated females. Clutch size was corrected for year, snout-vent length (SVL) and egg mass and postlaying mass for year and SVL prior to analysis. Note the statistically significant interaction term between female morphotype and clutch size; this reflects the morph-specific differences in slopes in the regressions of postlaying mass vs. clutch size (Fig. 5). Postlaying mass and clutch size were corrected for year and snout-vent length (SVL) prior to analysis. Type III sums of squares. Full model: $F_{6,64} = 5.458$; $R^2 = 0.338$; $P < 0.001$.

Source	d.f.	MS	F	P
Clutch size	1	0.050	0.237	0.63
Female morphotype	1	0.009	0.042	0.84
Experimental category	1	2.121	10.048	0.002
Morph × clutch size	1	0.854	4.048	0.048
Exp × clutch size	1	0.281	0.993	0.32
Exp × morph	1	0.210	2.728	0.1035
Residual	64	0.211		

Dependent variable: postlaying mass.

Table 3 Binomial linear model (probit link) for how field survival from the first to the second clutch (dependent variable) of female lizards is influenced by variation in antibody responsiveness, female morphotype (yellow = 0, orange = 1), experimental category (control = 0, follicle-ablation = 1) and all two-way interactions. Sample sizes: 47 control females and 30 follicle-ablated females. Note the statistically significant interaction term between female morphotype and antibody responsiveness; this reflects the morph-specific differences in slopes of the fitness functions (Fig. 6).

Source	d.f.	Wald statistics	P
Antibody response	1	1.3613	0.24
Female morphotype	1	1.5568	0.21
Experimental category	1	0.0001	0.99
Morph × antibody response	1	8.7040	0.003
Exp × antibody response	1	0.5687	0.45
Exp × morph	1	0.251	0.29

Dependent variable: field survival from first to second clutch.

Svensson *et al.*, 2001b). As the assumptions of parametric regression analysis may become violated when one is dealing with dichotomized variables like survival (non-normally distributed residuals), we analysed the survival effects using a binomial linear model with a probit link in the VGLZ module of STATISTICA (see Table 3). We also analysed our experimental results using path analysis, a complementary approach suitable for data that is structured in causal chains like in our study system (Svensson *et al.*, 2001a; see next section).

Path analysis of natural and experimental variation

We have previously used path analysis to reveal links between social environment, condition and survival for unmanipulated females (Svensson *et al.*, 2001a). Here we include data on experimentally induced reproductive variation, taking our previous basic model (Svensson *et al.* 2001a; Fig. 1) as our point of departure.

We estimated path coefficients and model fit using the SEPATH module in STATISTICA. Statistical fit of a particular path model was evaluated by comparing the observed covariance among variables to the expected covariance if the model was true. A significant difference between the observed and the expected covariance matrices indicates that a model has a poor fit (Mitchell, 1993). Following standard procedures (Gomez & Zamora, 2000), we first evaluated a full (complete) path model, and subsequently tested simpler models that were 'nested' within this basic model. These nested models were identical in causal structure and contained all the original variables, except that some (weak) paths were constrained to zero compared with the original (complete) model. We only present the 'best' models, that is, models with the lowest χ^2-value, largest P-value and greatest number of degrees of freedom (Mitchell, 1993). We incorporated experimental treatment as a dummy variable (control subject = 0; experimental = 1). We

(consisting of 13 from the follicle-ablation experiment and 16 from the corticosterone-experiment).

We analysed experimental and survival effects using general linear models with type-III sums-of-squares in the VGLM module in STATISTICA (Statsoft, 2000). We constructed models with three independent variables and all two-way interactions to investigate both main and interaction effects of the variables of interest (Tables 1–3). Of particular interest in this study are the interaction effects, because these may indicate differential responses to the social environment of the morphs, or when survival is the dependent variable: fitness epistasis and/or correlational selection (Whitlock *et al.*, 1995;

analysed the two experimental treatments, follicle ablation and B-implantation, in separate path models and compared differences in effects between the female morphs. We incorporated the number of orange and yellow neighbours as a measure of the local social environment a female encounters during the production of her clutch.

For the B-implanted females, territory information was not available and hence was not incorporated in the analyses. Instead we incorporated laying date as a variable in the B-experiment, because we knew from previous experimental studies that this type of manipulation delays laying date of the first clutch (Sinervo & DeNardo, 1996). In contrast, laying date was not incorporated in the path model for the follicle ablation experiment, as such this experimental manipulation has no effect on laying date (Sinervo & DeNardo, 1996).

Finally, to compare the overall differences between yellow and orange females in their responses to our experimental manipulations and to variation in the social environment, we calculated the correlation matrices of traits, environmental variables and experimental treatments between the two morphs. We tested for equality of these matrices using a maximum-likelihood test in STATISTICA (Jennrich, 1970). A significant difference (high χ^2-value), would indicate that the matrices differ between the two morphs, and hence yellow and orange females respond differently to variation in social environment and to our experimental manipulations.

Results

General effects of follicle-ablation experiment

As in our previous studies using the follicle-ablation experimental procedure, experimental females laid significantly smaller clutches of larger eggs (Figs 1 & 2). The effects were most pronounced among the yellow females, but similar, albeit weaker, trends were found in the orange females (Figs 1 & 2).

Effects of the social environment

The social environment had a strong impact on immunocompetence in the two morphs. Immunocompetence was significantly reduced with increasing number of orange neighbours among orange females whereas there was no such effect in yellow females (Fig. 3). Thus, the immunocompetence reaction norms towards the social environment differed between the two morphs, as revealed by a statistically significant interaction term between female morphotype and number of orange neighbours (Table 1). The experimental treatment did not confound these results, as indicated by the absence of a significant experiment × orange neighbour interaction (Table 1). Closer inspection of Fig. 3 reveals that the morphs appeared to be nonrandomly distributed with

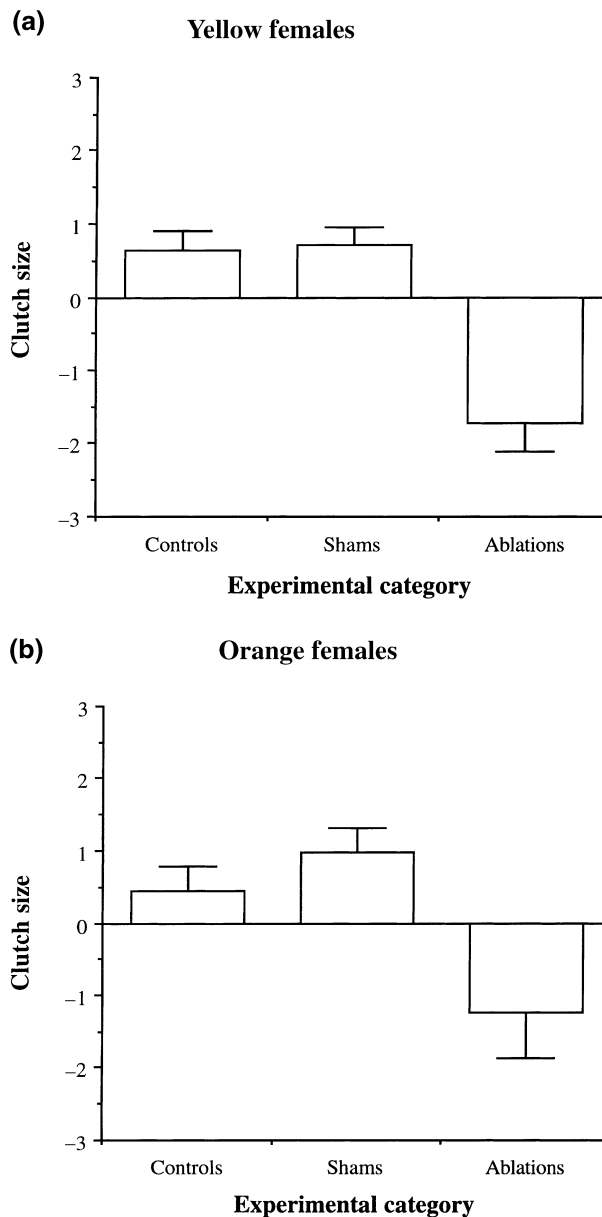**(a)**

Yellow females

(b)

Orange females

Fig. 1 Results of follicle ablation experiment on clutch size (residuals) in female lizards. Means (±SE) are shown for control females, sham-manipulations and ablated females. Data from 1998 and 1999 was corrected for year prior to analysis. (a) *Yellow females*: The difference between the female categories is significant: $F_{2,58} = 22.458$; $P < 0.001$. Differences between the ablation group and the shams, and the ablation group and controls are both significant (Fisher's protected LSD: both P's < 0.001). Difference between sham and controls is not significant (Fisher's protected LSD). (b) *Orange females*: The difference between female categories is significant: $F_{2,29} = 11.623$; $P < 0.001$. Fisher's protected LSD: ablations vs. controls: $P < 0.001$; ablations vs. shams: $P < 0.05$; controls vs. shams: n.s.
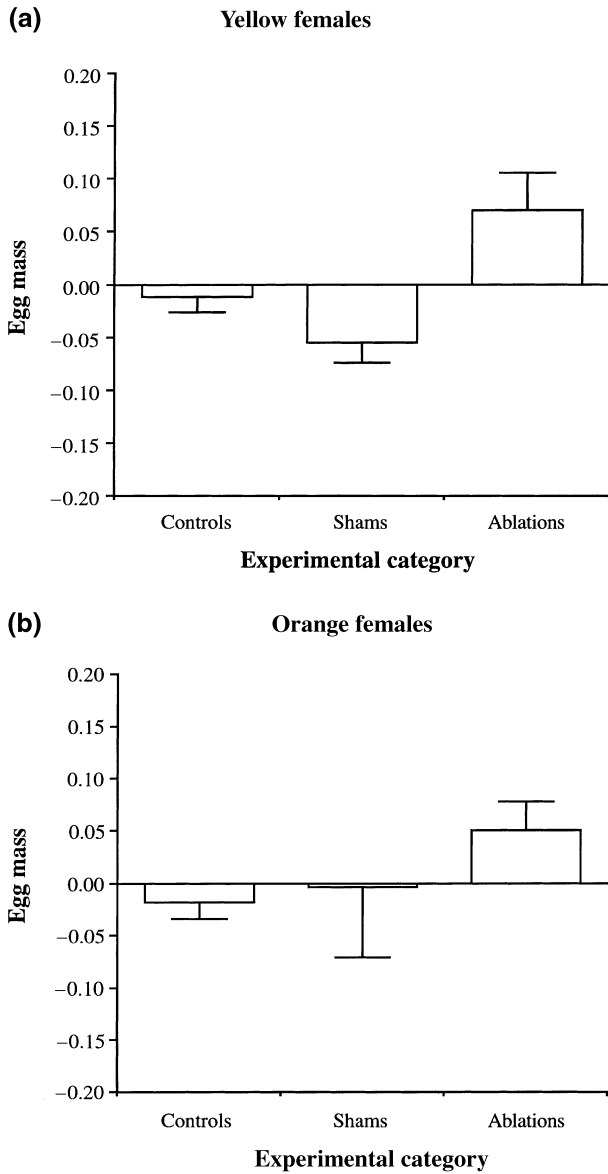
(a)

Yellow females

(b)

Orange females

Fig. 2 Results of follicle ablation experiments on egg mass (residuals) in female lizards. Means (±SE) are shown for control females, sham-manipulations and ablated females. Data from 1998 and 1999 was corrected for year prior to analysis. (a) *Yellow females:* The difference between the female categories is significant: $F_{2,56} = 6.328$; $P < 0.001$. Differences between the ablation group and the shams, and the ablation group and controls are both significant (Fisher's protected LSD: ablations vs. controls: $P < 0.01$; ablations vs. shams: $P < 0.01$). Difference between sham and controls is not significant (Fisher's Protected LSD: n.s). (b) *Orange females*: The difference between the female categories is significant: $F_{2,26} = 7.977$; $P < 0.01$. Fisher's protected LSD: ablations vs. controls: $P < 0.001$; ablations vs. shams: n.s.; controls vs. shams: n.s.

respect to social environments, in that orange females are more often found solitary or with only one orange neighbour than are yellow females. As this effect may
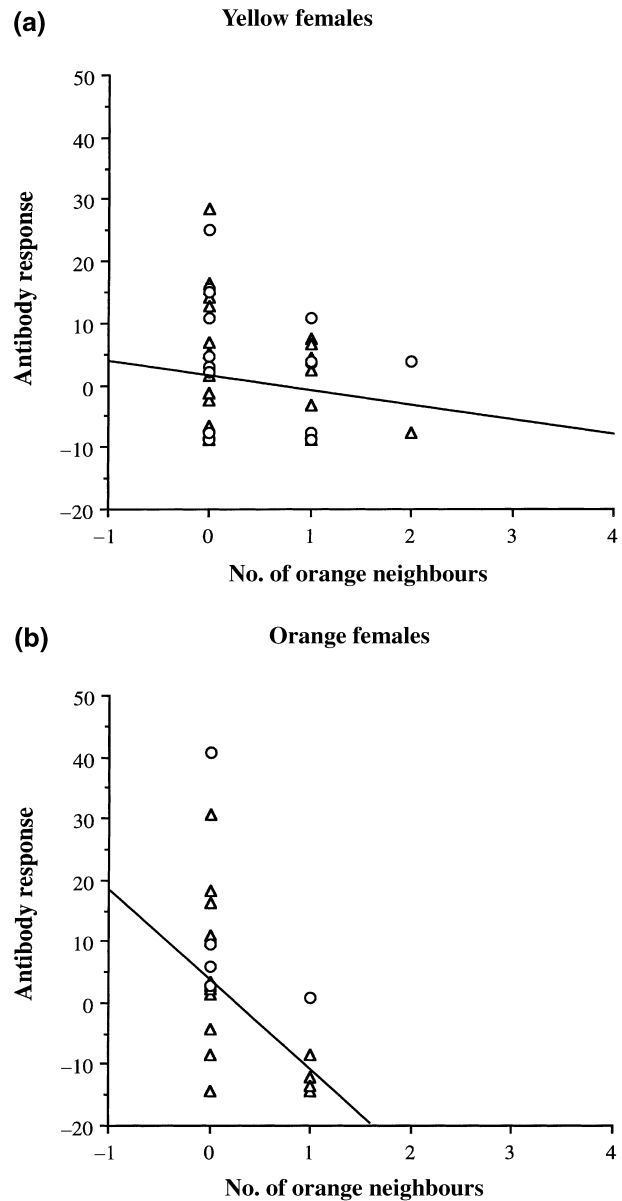
(a)

Yellow females

(b)

Orange females

Fig. 3 Relationship between antibody responsiveness and number of orange neighbours for female lizards. Circles: Follicle-ablated females. Triangles: Control females. (a) *Yellow females:* There is no effect of the number of orange neighbours and a significant positive effect of the manipulation (orange neighbours: $F_{1,46} = 0.002$, $P = 0.96$; experiment: $F_{1,46} = 4.631$, $P < 0.05$). Equation for the regression line: $Y = -4.386 + 0.567X$, n.s. ($n = 49$). (b) *Orange females:* There is a negative effect of the number of orange neighbours but no significant effect of the experimental manipulation (orange neighbours: $F_{1,24} = 6.887$, $P < 0.05$; experiment: $F_{1,24} = 1.147$, n.s.). Equation for the regression line: $Y = 3.978 - 15.669X$, $P < 0.01$ ($n = 26$).

bias the statistical tests, we recalculated the interaction effect we found (morph × orange neighbours; Table 1), using a categorical measure of the number of orange

neighbours instead (presence / absence of orange neighbours). The interaction effect was still significant ($F_{1,72} = 7.082$; $P < 0.01$), indicating that the stronger social sensitivity of orange females was an artefact of biases in the social environments encountered.

In contrast, there were no effects of the number of yellow neighbours on the immunocompetence of either morph (both P's > 0.10), and there were also no effects of the number of orange or yellow neighbours on postlaying mass in neither orange nor yellow females (all P's > 0.10).

Effects of follicle-ablation experiment on condition

As a result of the experimentally reduced clutch sizes, the phenotypic variance for this trait increased (Figs 1 & 2). We utilized this increased variation to investigate the impact of clutch size and egg mass on the two condition traits. As clutch size and egg mass are strongly correlated, we removed the effects of egg mass from clutch size and the effects of clutch size from egg mass using residuals from linear regressions. These analyses revealed that immunocompetence was significantly reduced with increasing clutch size (Fig. 4). The data from both orange and yellow females were combined in Fig. 4 as there was
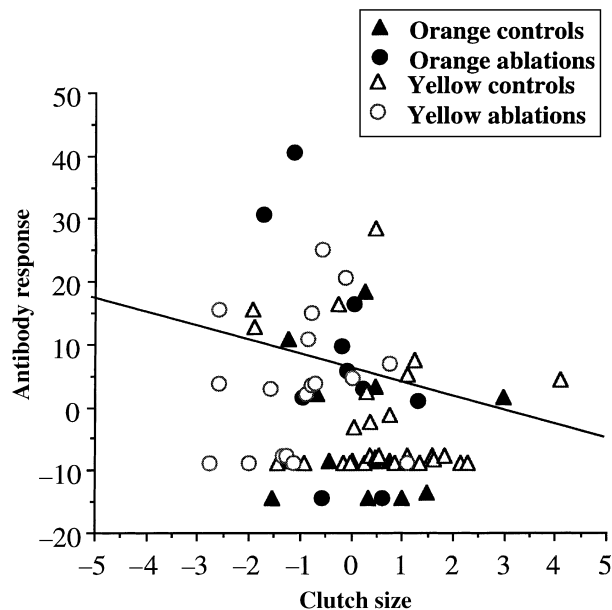

Fig. 4 Antibody responsiveness vs. clutch size (natural variation and experimentally induced variation from follicle-ablation experiments). Both female morphs were combined and we did not find any difference between these two types of females (see Results). Circles: Follicle-ablated females. Triangles: Control females. The negative relationship is significant: $F_{1,71} = 4.211$; $R^2 = 0.056$; $P < 0.05$. Equation for the regression line: $Y = -2.842 - 2.275X$. There was no significant remaining difference between the experimental categories that was not attributable to their differences in clutch size (see Results).

no indication that the relationship differed between the two female morphs (morph: $F_{1,68} = 1.728$, n.s.; throat × clutch size: $F_{1,68} = 0.493$, n.s.). There was no evidence that surgical manipulation *per se* had any impact on immunocompetence when the effects of clutch size, experimental treatment and number of orange neighbours were included in a common analysis (clutch size: $F_{1,63} = 4.346$, $P < 0.05$; experiment: $F_{2,63} = 1.359$, $P = 1.965$, n.s.; orange neighbours: $F_{1,63} = 4.319$, $P < 0.05$; all two-way interactions: $P > 0.05$). Egg mass did not have any significant effect on immunocompetence in yellow ($F_{1,45} = 1.134$, n.s.) or orange females ($F_{1,23} = 0.452$, n.s.), and there was no evidence that the relationship differed between the two morphs (morph × egg mass: $F_{1,68} = 1.174$, n.s.).

Clutch size had strong and negative effect on postlaying mass in yellow females, compared with the absence of such a relationship among orange females (Fig. 5a,b). Thus, reproductive investment reduced postlaying mass to a greater extent in yellow than orange females. This is revealed by the statistically significant interaction term between female morphotype and clutch size which shows that the slopes relating postlaying mass to clutch size was steeper for yellow than orange females (Fig. 5a. b; Table 2).

Condition traits and survival

Variation in immunocompetence affected field survival in both morphs, but in different ways (Figs 6 & 7; Table 3). In yellow females, there was a significant positive relationship between antibody responsiveness and survival (Fig. 6a), whereas in orange females there was a more complicated relationship, as shown by 'hump' at low antibody response values and declining survival at higher values (Fig. 6b). The difference in slope of the two morph-specific fitness functions was significant, and the effect was not confounded by survival effects mediated via the experimental manipulation *per se* (Table 3). The effects of postlaying mass on survival was much weaker and not significant for either morph (P-levels for all main and interaction effects > 0.10; data not shown).

Summary of follicle ablation experiment

We have summarized our findings above and in Figs 1–6 in separate path diagrams for the two female morphs (Fig. 7a,b). The model fit for the orange females was good ($\chi^2_{|16|} = 18.89$, $P = 0.27$), whereas the model fit for the yellow females was not as good as revealed by a significant discrepancy between the observed and the expected covariance matrices ($\chi^2_{|17|} = 30.41$, $P = 0.023$).

To study the difference between the two morphs and their response to our experimental manipulations, we tested if the correlation matrices containing all the variables in Fig. 7 (eight variables) differed between
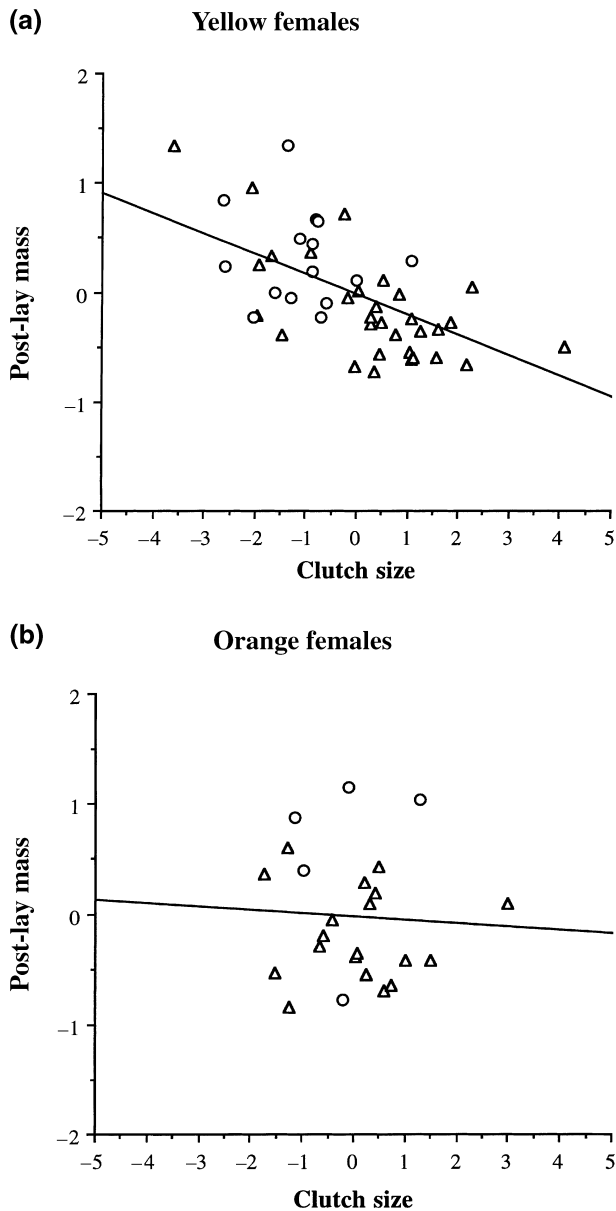
(a)

Yellow females

(b)

Orange females

Fig. 5 The relationship between postlaying mass and clutch size (natural and experimental variation). Clutch size was corrected for year, snout-vent length (SVL) and egg mass, and postlaying mass for year and SVL prior to analysis. Circles: follicle-ablated females. Triangles: control females. (a) *Yellow females:* There is a significant negative effect of clutch size and a slight additional effect of the manipulation [clutch size: $F_{1,43} = 14.401$, $P < 0.001$; experiment: $F_{1,43} = 2.913$, $P = 0.095$. Equation for the regression line: $Y = -0.022 - 0.197X$, $P < 0.001$ ($n = 46$)]. (b) *Orange females:* There is no significant relationship between clutch size and postlaying mass, but a significant effect of the manipulation (clutch size: $F_{1,22} = 0.259$, n.s.; experiment: $F_{1,22} = 4.524$; $P < 0.05$). Equation for the regression line: $Y = -0.031 - 0.024X$, n.s. ($n = 24$). The slopes differ significantly between the two morphs (Table 2).

orange and yellow females. The two correlation matrices indeed differed significantly [$\chi^2_{|28|} = 62.93$, $P < 0.001$, maximum likelihood procedure (ML) in STATISTICA], suggesting that the two morphs responded differently towards the social environment and our experimental manipulations, and that the fitness effects also differed between yellow and orange females.

Corticosterone-experiment

We present the results of our B-implant experiment in morph-specific path diagrams in Fig. 8. We incorporated laying date as an additional variable in this model to account for the significant effects of B-manipulation on this trait (Sinervo & DeNardo, 1996; Fig. 8). Again, we present the best models, starting from a basic ('complete') path model that was subsequently simplified by constraining weak paths to zero. The fit of the models illustrated in Fig. 8 were good, both for yellow females ($\chi^2_{|9|} = 5.04$, n.s.) and for orange females ($\chi^2_{|12|} = 11.46$, n.s.).

Strong and positive effects of corticosterone on immunocompetence in both yellow and orange females were present (Fig. 8a,b). These effects contradict the traditional view of this hormone as being primarily immunosuppressive (see Sapolsky, 1992 for discussion). To check if the immunoenhancing effects we observed were due to the effects of corticosterone and not because of the surgical manipulation *per se,* we investigated how antibody responsiveness was influenced by year, experimental category (controls, shams and B-implants) and clutch size. This analysis revealed that experimental category had a strong and significant effect on antibody responsiveness, whereas the other factors did not (year: $F_{1,80} = 1.597$, n.s.; experimental category: $F_{2,80} = 11.560$, $P < 0.001$; clutch size: $F_{1,80} = 0.445$, n.s.). The B-implants had higher antibody titres than sham-manipulated and control females [LS means (±SE): B-implants: 23.11 ± 2.94; shams: 9.79 ± 3.83; controls: 5.80 ± 2.10]. *Post-hoc* tests revealed that the differences between both B-implants and shams and B-implants and controls were both significant, whereas there was no significant difference between shams and controls (Fisher's LSD: B-implants vs. controls: $P < 0.001$; B-implants vs. shams: $P < 0.01$; controls vs. shams: n.s.).

In addition to the effects of the B-implant on immunocompetence, there were multiple, 'pleiotropic' effects of this manipulation on lay date, egg mass and postlaying mass (Fig. 8). Note especially that in the orange females, B-implantation had two opposite effects on postlaying mass: another via a direct and one via an indirect pathway (Fig. 8b). The manipulation had a direct and positive effect on postlaying mass, and one negative, indirect effect via delayed lay date, which in turn reduced postlaying mass (Fig. 8b). Thus, orange females gained in postlaying mass from the B-manipulation, but this effect was partly cancelled out by the later start of egg laying
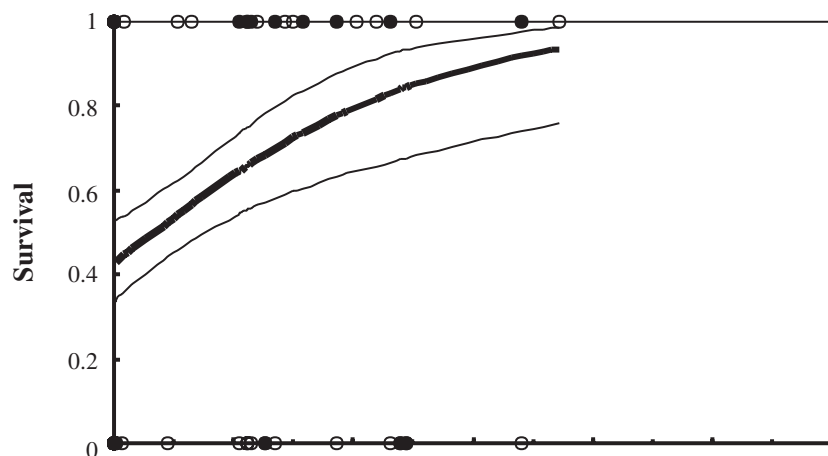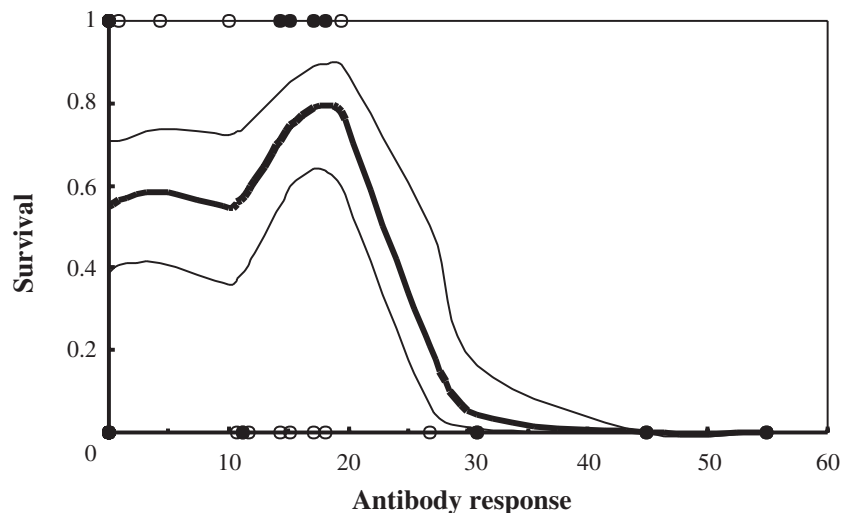
(a) Yellow females

(b) Orange females

Fig. 6 Fitness functions (field survival from first to second clutch) showing how survival is affected by variation in immunological condition (antibody responsiveness). Fitness functions (means ± SE's) were calculated using nonparametric cubic spline regression (Schluter, 1988). Solid points: follicle-ablated females. Open points: control females. Survival effects tested with Likelihood Type 3 tests. Note positive relationship between antibody response in yellow females and negative relationship in orange females. (a) *Yellow females: Antibody response*: Log-likelihood $= -33.328$, $\chi^2 = 9.281$, $P < 0.01$; *Experimental category*: Log Likelihood $= -28.689$, $\chi^2 = 0.001$, n.s.; *Year*: Log-likelihood $= -31.815$, $\chi^2 = 6.253$, $P < 0.05$. (b) *Orange females: Antibody response*: Log-likelihood $= -17.607$, $\chi^2 = 3.391$, $P = 0.066$; *Experimental category*: Log-likelihood $= -16.523$, $\chi^2 = 1.223$, n.s.; *Year*: Log-likelihood $= -16.230$, $\chi^2 = 0.636$, n.s.

among these females which in turn reduced postlaying mass (cf. Schluter & Gustafsson, 1993 their Fig. 3).

Finally, we tested if the correlation matrices between yellow and orange females differed also in the corticosterone-experiment, as found in the follicle-ablation experiment. This was indeed the case, although the discrepancy between the matrices of orange and yellow females was not as large as in the follicle-ablation experiment, as judged by higher probability value in the significance test ($\chi^2_{|21|} = 38.09$, $P < 0.05$). This indicates that the corticosterone-experiment had a more similar effect on the two morphs than the follicle-ablation experiment. Alternatively, omission of information about the social environment, may have reduced some of the differences between the two morphs (cf. Fig. 7).

Discussion

Importance of interaction effects

Particularly striking results in this study are the *interaction effects* between female morphotype, traits, social environments and survival (Figs 3, 5 & 6; Tables 1–3). These interaction effects reveal the existence of different adaptive character combinations between morphotype and other fitness-related traits (Fig. 6) and that morphs respond differently to local social environments (Fig. 3). Morphs occupy different 'adaptive peaks' (Wright, 1932), and the existence of several different optimal character combinations allow them to solve similar ecological problems in different ways (cf. Whitlock *et al.*, 1995).
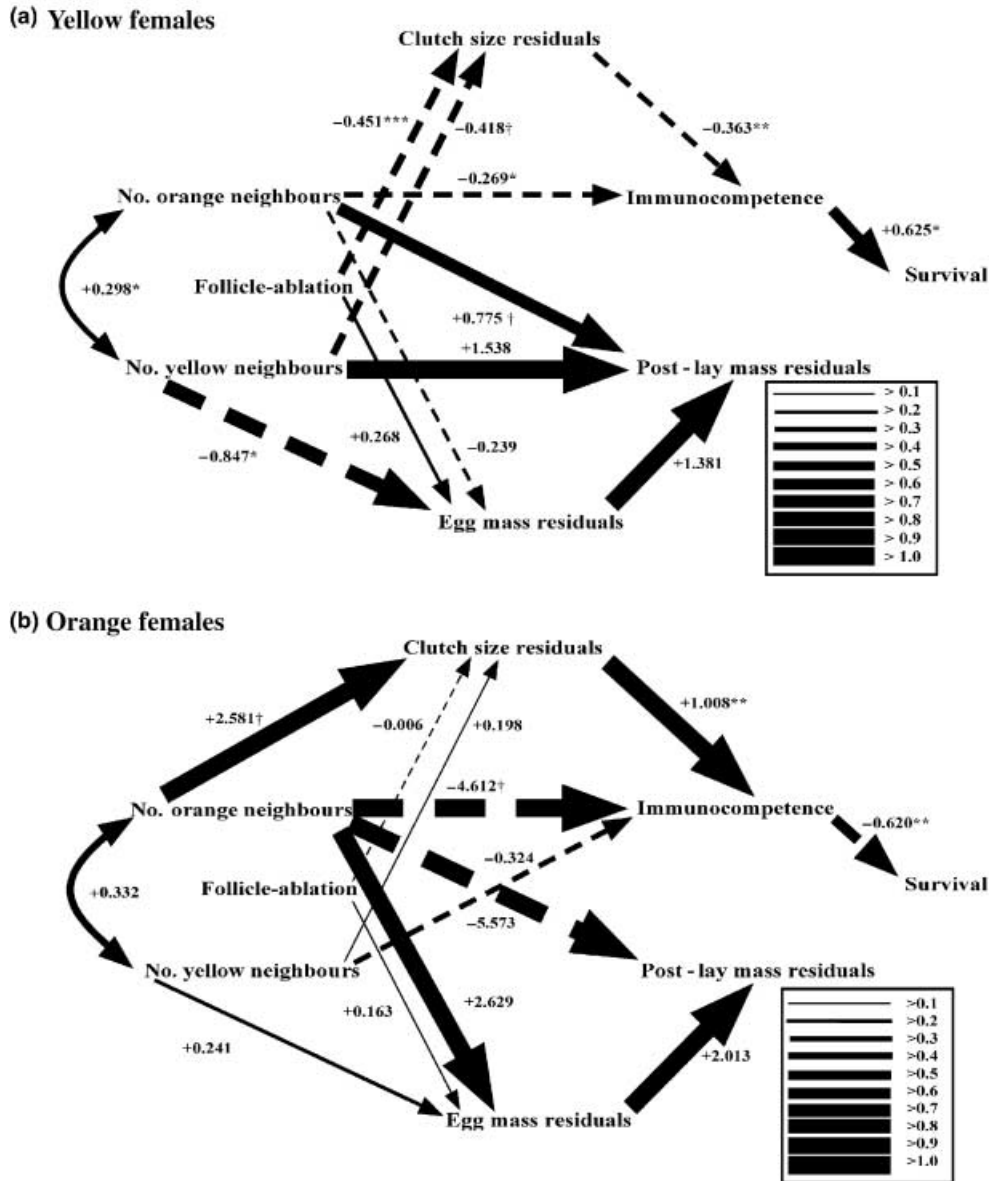
Fig. 7 Path diagrams showing how the social environment (number of orange or yellow neighbours) influences life-history traits (clutch size, egg size) and condition traits (immunocompetence, postlaying mass) among the two female lizard morphs. Thickness of paths indicate absolute magnitude of path coefficients. Solid lines: positive path coefficients. Dashed lines: negative path coefficients. We also show variation in condition traits in turn affects survival. In the path diagrams, we have also incorporated our experimental treatment (follicle ablation) and its effects on clutch size and egg size. All variables were corrected for year and standardized to mean zero and unit variance prior to analysis. Clutch size, egg size and postlaying mass were corrected for snout-vent length (SVL) prior to analysis. For the purpose of clarity, we have omitted correlations between immunocompetence and postlaying mass and between clutch size and egg mass, as well as all residual terms ($U's$). For quantitative graphs of particular paths; see Figs 1–6. (a) *Yellow females:* Note especially the weaker effects of the number of orange neighbours, the strong and negative effects of clutch size on immunocompetence and the strong positive effect of immunocompetence on survival. (b) *Orange females:* Note especially the strong effect of the number of orange neighbours on clutch size, egg size, immunocompetence and postlaying mass and the negative effect of immunocompetence on survival (cf. 7a). Symbols: ***$P < 0.001$; **$P < 0.01$; *$0.01 < P < 0.05$; [†]$0.05 < P < 0.10$.

Experimental perturbations of the different morphs displace them from their adaptive peaks and can hence reveal both the physiological mechanisms underlying the coadapted trait complexes in the morphs and the correlational selection pressures that operate to maintain and refine the integrity of the different phenotypes.

(a) Yellow females
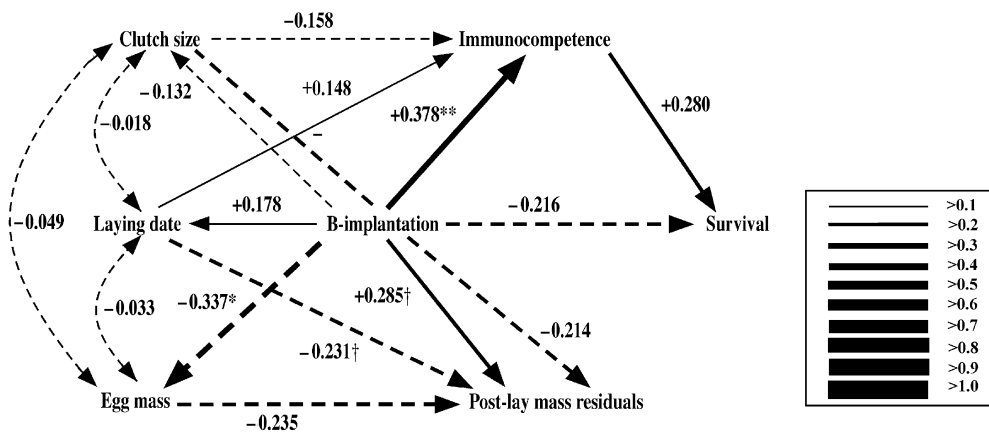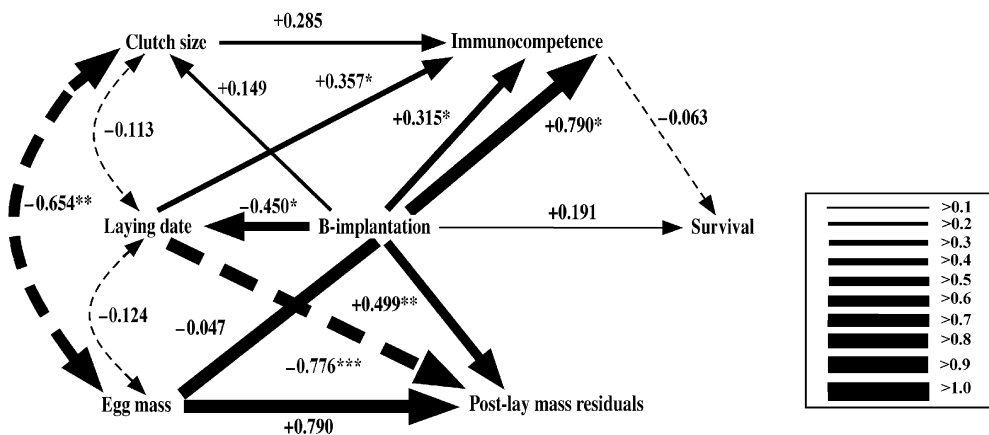

(b) Orange females

Fig. 8 Path diagrams demonstrating the pleiotropic effects of corticosterone experiment (B-implantation) on reproductive traits (clutch size, egg mass and laying date), condition traits (postlay mass and immunocompetence) and survival. Solid lines: positive path coefficients. Dashed lines: negative path coefficients. All variables were corrected for year and standardized to mean zero and unit variance prior to analysis. Unmanipulated females: 0. B-implanted females: 1. Clutch size, egg size and postlaying mass were also corrected for snout-vent length (SVL) prior to analysis. Notice that these path diagrams differ from those in Fig. 7 in that we have not incorporated measurements of the social environment here (not available) and clutch size and egg mass have not been corrected for each other (as evident by double-headed arrows showing correlations between these two traits). For the purpose of clarity, we have omitted correlations (double-headed arrows) between immunocompetence and postlaying mass, as well as residual terms (*U*'s). (a) *Yellow females:* Note especially the strong positive effect of the corticosterone-treatment on immunocompetence (positive) and the negative treatment effect on egg size. (b) *Orange females:* Note especially the positive corticosterone-effect on laying date, the negative path from laying date to postlaying mass and the positive direct path from treatment to postlaying mass. Symbols: ***$P < 0.001$; **$P < 0.01$; *$0.01 < P < 0.05$; †$0.05 < P < 0.10$.

The epistatic fitness effects of female morphotype and immunocompetence should lead to a buildup of linkage disequilibrium between the alleles for morphotype and those for immune function, even if these traits are governed by separate sets of loci (Lynch & Walsh, 1998; Sinervo & Svensson, 2002). If correlational selection is strong relative to recombination, and aided by physical linkage of the loci and/or close inbreeding in the population, a genetic correlation can then be formed between the interacting fitness-traits (Lande, 1980, 1984). We have evidence for such a genetic correlation because orange females produced daughters with low antibody responses and yellow females daughters with high antibody responses (Svensson *et al.*, 2001b).

The two integrated phenotypes may largely be shaped by epistatic selection in which alleles for high antibody responsiveness are selected against in genetic backgrounds of orange females, but are favoured in genetic backgrounds of yellow females. The multilocus polymorphism in throat colour and immunocompetence is in turn likely to be maintained by frequency-dependent selection driven by continually evolving and changing social environments, parasites and infectious diseases (Sinervo et al., 2000a; Svensson et al., 2001b). Strong genetic correlations between throat colour and reproductive traits (r_A-estimates ranging from -0.84 to 1.09) further point to an important role for correlational selection in the buildup and maintenance of multitrait differences between these morphs (Sinervo & Svensson, 2002).

Physiological manipulations and their consequences

If morphs are occupying different adaptive peaks, as we have argued above, and the different optimal trait combinations partly result from pleiotropic effects of steroid hormones such as progesterone or corticosterone (Moore et al., 1998), one should ideally manipulate the levels of these hormones and investigate the different effects on condition in the different morphs. One should also investigate how variation in condition affects morph-specific survival. As the two female colour morphs are selected towards different clutch size, egg mass and immunocompetence optima (Sinervo et al., 2000a), manipulations of clutch size and egg mass should have different fitness consequences in orange and yellow females, as we have indeed found (Figs 6 & 7).

Interestingly, manipulations of clutch size using follicle-ablations had opposing effects on immunological condition in orange and yellow females, and immunological condition in turn had different fitness effects in the two morphs (Figs 6 & 7). These data suggest that the two female morphs are functionally integrated phenotypes that are selected towards different optimal trait combinations of morphology, physiology and behaviour (see also Sinervo et al., 2000a; Svensson et al., 2001a). We suggest that differences in habitat selection and thermal conditions may select for different optimal investment in immune function in orange and yellow females. Orange females tend to occupy hotter, drier habitats than yellow females (B. Sinervo, E. Svensson & T. Comendant, unpublished observations) and the relative mortality rates caused by parasitic infections may differ between such environments, which in turn may drive correlational selection between morphotype and immunological condition (Fig. 6).

Our findings of how experimentally increased corticosterone enhanced immunocompetence (Fig. 8) are surprising and may at first sight contradict the conventional view of corticosterone as being immuno-suppressive (Sapolsky, 1992; Wedekind & Folstad, 1994). However, corticosterone has many effects on physiology apart from the effects on immune function (Wingfield et al., 1998). Wingfield et al. (1998) argued that corticosterone is a major life-history hormone that mediates trade-offs between the conflicting demands of current reproduction, condition and future survival. Corticosterone enhances blood glucose levels (Sapolsky, 1992; Wingfield et al., 1998), and this may improve several aspects of condition, including immunocompetence. Corticosterone also interacts antagonistically with both testosterone (T) and progesterone (P), both of which are hormones that may cause immunosuppression (Cooper & Greenberg, 1992; Folstad & Karter, 1992; DeNardo & Sinervo, 1994a, b; Moore et al., 1998).

Path analysis, experiments and interaction effects

We have already discussed the morph-differences in the signs of the paths between the clutch size, immunological condition and future survival (Fig. 7). Paths that have different signs in different path diagrams is another way of demonstrating interaction effects in more conventional analysis of variance (Tables 1–3). Two other important pieces of information can be inferred from the path diagrams. First, when there are both direct and indirect effects of an experimental manipulation, it is quite possible that these effects can have opposite signs and hence counteract each other. An example of this may be the positive direct path following B-manipulation on orange female's postlaying mass, which is counteracted by a negative, indirect path on postlaying mass via its effect in delaying lay date (Fig. 8b). Secondly, from our path diagrams we can also quantify the strength of both direct and indirect experimental effects. In yellow females, there are strong and significant paths from experimental manipulation to clutch size, from clutch size to immunocompetence and from immunocompetence to survival (Fig. 7a). By reducing clutch size using follicle-ablations we enhanced immunocompetence, and this in turn had a positive effect on the yellow female's field survival. The experimental effect on survival from the follicle-ablation experiment can be quantified by multiplying the successive path coefficients in the chain: $(-0.451) \times (-0.363) \times (0.625) = 0.102$. Interestingly, our findings for orange females are quite different than those for yellow females (Fig. 7b). Although there was no significant effect of follicle-ablation on clutch size (-0.006), there were significant effects of clutch size on immunocompetence ($+1.008$) and from immunocompetence on survival (-0.620) (Fig. 7b). Note that these paths are opposite in sign compared with the same paths in yellow females (Fig. 7a,b). Again, this illustrates interaction effects and the multifarious differences in physiology and reproductive traits between the two phenotypes.

Conclusions

The results in this study have several general implications that are likely to be important in other study systems. First, different genotypes (e.g. morphs) may respond differently to local (social) environments. Secondly, the fitness consequences of variation in condition traits may often differ between genotypes, leading to correlational selection. Thirdly, variation in reproductive allocation between genotypes and its adaptive significance can be fruitfully investigated by the use of physiological manipulations that should lead to different fitness consequences in different genetic backgrounds. Understanding the relative importance of genetic and environmental factors influencing condition and in turn the adaptive significance of variation in condition is a major challenge for evolutionary biologists (Houle, 1991; Rowe & Houle, 1996).

Acknowledgments

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