

# Testing the status-dependent ESS model: population variation in fighter expression in the mite *Sancassania berlesei*

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## Keywords:

alternative reproductive strategies;  
male polyphenism;  
*Onthophagus taurus*;  
phenotypic plasticity;  
reaction norm.

## Abstract

The conditional evolutionarily stable strategy (ESS) with status-dependent tactics is the most commonly invoked ESS for alternative reproductive tactics within the sexes. Support for this model has recently been criticized as apparent rather than real. We address key predictions of the status-dependent ESS in three populations of the male dimorphic mite *Sancassania berlesei*. In *S. berlesei* 'fighter' males are characterized by a thickened pair of legs used for killing rivals; 'scramblers' are benign. Most males in each population could be manipulated to become fighters by decreasing density, fulfilling the prediction that males make a 'decision'. There was evidence of genetic covariance between sire status and offspring morph, but also a strong effect of sire morph on offspring morph ratio. This was consistent with considerable genetic variation for the status-dependent switch point as a breeding experiment found no support for single-locus inheritance. We also found evidence that switch points evolve independently of distributions of status. This study supports the current status-dependent ESS model.

## Introduction

Within many species, males exhibit alternative reproductive tactics (Shuster & Wade, 2003). In species in which there is no evidence for a genetic polymorphism in male tactics (Maynard Smith, 1982), the tactics are thought to be assigned probabilistically under a mixed evolutionarily stable strategy (ESS) or reflect state or status-dependent selection manifested as a conditional ESS (Gross, 1996). Frequently alternative reproductive tactics are accompanied by alternative morphologies, reflecting the morphological selective optima of the alternative tactics. For example, horned and 'hornless' onthophagine dung beetles, respectively adopt guarding and sneaking tactics (Emlen, 1997; Hunt & Simmons, 1998; Moczek & Emlen, 2000).

The evolution of the conditional ESS with status-dependent male reproductive tactics has been reviewed

by Gross (1996), with additional modelling of the inheritance in this ESS by Hazel *et al.* (1990) and Gross & Repka (1998). In the model, status (e.g. body size) is normally distributed and hypothetical linear fitness functions of the alternative tactics are represented with different slopes. The consequence of divergent slopes of the fitness functions is that fitness functions can intersect, such that there is a transition with increasing or decreasing status from a higher fitness return for one tactic, to a higher fitness return for the alternative (Gross, 1996). The model predicts that individuals will adopt the phenotype from which they will derive the highest fitness return for their status, hence, the intersection of the fitness functions translate phenotypically as a shift from one tactic to another across a threshold status (Gross, 1996). The fitness functions of the tactics determine where the threshold (ESS switch point) lies in relation to the status distribution of the population, and therefore the ratio of the alternative tactics in the population (Gross, 1996). Such morphological and behavioural diversity within a sex, occurring in the absence of a simple genetic polymorphism, represents an extreme case of phenotypic plasticity (West-Eberhard, 2003).

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Gross (1996) set out five key predictions of the status-dependent ESS model. First, that the tactics involve a 'decision' by the individual. Secondly, that the decision is made relative to some aspect of status. Thirdly, that individuals are genetically monomorphic for the decision. Fourthly, as outlined above, individuals adopt the tactic from which they will derive the highest fitness benefit, and finally, that the fitness of the alternative tactics are not equal, except at the switch point status (Gross, 1996). Here we address the first three of these predictions. These three requirements: that individuals make a 'decision', that the decision is relative to some measure of status and that the population is monomorphic with respect to the decision, cannot easily be demonstrated. For example individuals need to adopt a tactic in a status-dependent manner, but in order to satisfy the criterion of a decision, it must be shown, that by altering the hypothesized fitness benefit to the individual, that the decision can be altered. Shuster & Wade (2003) have recently argued that in many cases it is only part of the population that is responding to environmental cues, hence that the decision is not made by all individuals, but only a few, and dependent upon their genetic background. That decisions are made in a status-dependent way is relatively easy to demonstrate in the context of behavioural tactics (e.g. Forsyth & Alcock, 1990). The requirement that all individuals are making a decision (i.e. are capable of expressing either phenotype) and those decisions are conditional on status is more difficult to satisfy in species with alternative male morphologies. This requires status to be directly manipulated (e.g. Radwan, 1995; Hunt & Simmons, 1997; Moczek & Emlen, 1999; Tomkins, 1999; Radwan *et al.*, 2002).

The expectation that individuals are monomorphic for the decision has been questioned by Shuster & Wade (2003), who suggest that this is a major flaw in the status-dependent model. Shuster & Wade's (2003) criticism is based on the notion that 'genetic monomorphism' means that there is no genetic variance for the threshold, or for status. Rather than being overlooked however, these aspects of inheritance of the conditional strategy are outlined by Hazel *et al.* (1990) and Gross & Repka (1998; see also, Hazel & Smock, 2000), and are key components of the status-dependent model. In fact what 'monomorphism' means in the context outlined in the model, is that individuals have a genetic decision rule that enables them to express either male morph, i.e. to exhibit a conditional or status-dependent polyphenism (West-Eberhard 2003). Although the terminology is perhaps confusing, it was used as to make the distinction between the conditional strategy and the alternative strategy model, in which there is, a single locus genetic *polymorphism* for male morph, such that (at its simplest) individuals either have the allele to produce one morph, or the other (Gross, 1996). In order to test for polyphenism it has to be shown that males can produce either

phenotype by manipulating the environment in which they develop. Typically such manipulations have been very successful in inducing tactics that arise as a consequence of nutritional deprivation (Radwan, 1995; Hunt & Simmons, 1997; Moczek & Emlen, 1999; Tomkins, 1999). Inducing 'high' phenotypes (e.g. major males) in these same studies tends to have proven more difficult to achieve, and also interpret. When 'low' phenotypes are produced under 'high' conditions it is not possible to tell whether these males were genetically incapable of producing the high morph, or whether the experimental manipulation was at fault. For this reason the mode of inheritance of male morphs in species/populations where this occurs requires genetic analysis.

In addition to these three key predictions of the status-dependent model we address two additional hypotheses. The first relates to the inheritance of the male dimorphism. Threshold traits have long been modelled as being inherited through an underlying 'heritability of liability' (Falconer & Mackay, 1996; Roff, 1996, 1997; Lynch & Walsh, 1998) and contrary to Shuster & Wade (2003) we consider the liability model to have been implicit in the conditional strategy (e.g. Hazel *et al.*, 1990; Gross & Repka, 1998; Hazel & Smock, 2000). Hence, we test for the presence of an underlying liability in morph determination. Currently there is weak evidence for the heritability of body size in male dimorphic species (Emlen, 1996; Moczek & Emlen, 1999; Tomkins & Simmons, 1999; Kotiaho *et al.*, 2003), and few studies have yet formally addressed the heritability of alternative mating tactics in terms of the 'liability' model (Hazel *et al.*, 1990; Radwan, 1995; Gross & Repka, 1998).

The final hypothesis concerns switch point evolution. Status is a population specific parameter – an individual of high status in one population may be of low status in another, depending on the difference in mean competitive ability (e.g. size) between the populations (Gross, 1996). This means that the position of the switch point in a population is determined by the selection operating within the population (Gross, 1996). Populations are therefore expected to evolve unique morph ratios dependent upon the local historical, demographic and environmental factors that influence the slopes and elevations of the fitness functions within the population. Evidence for the independent evolution of male dimorphic switch points and status have so far been documented only in the earwig *Forficula auricularia* (Tomkins, 1999) and the dung beetle *Onthophagus taurus* (Moczek *et al.*, 2002).

Many species of Acarid mites have male polymorphisms, typically characterized by a thickened and sharply terminating third pair of legs in the 'fighter' morph. The Acarid mites are particularly interesting from the point of view of the evolution of male dimorphisms, because in some species there is a strong genetic component to morph determination, while others are largely environmentally determined (Radwan, 1995,

2001; Radwan *et al.*, 2002). Here we have tested the hypotheses set out above using three populations of the male dimorphic mite *Sancassania berlesei*. *Sancassania berlesei* is an ideal study species for investigating the decision making process in a dimorphic species, due to the ease with which the male dimorphism can be manipulated. Unlike dimorphic beetles and earwigs in which the switch point is more-or-less set at a particular body size, in *S. berlesei*, males express the fighter phenotype when they are reared at low density and the scambler phenotype when they are reared at high density. At intermediate densities the morphs are expressed in an apparently status-dependent manner – at least in one population (Radwan *et al.*, 2002). The density dependence of fighter expression is mediated through chemicals emanating from the colony. Here we test the key predictions of the status-dependent ESS by examining population variation in morph expression.

## Methods

### Collection and maintenance of populations

Three populations were used in these experiments, 'Stirling', 'Balmerino' and 'Germany'. The Stirling population was collected near Stirling central Scotland in 1998 and the Balmerino population from near a village of that name in Fife, Scotland in 2001. Both populations were collected from chicken-farm litter and have been maintained in mass culture since collection. The 'Germany' population was obtained from Department of Morphology, University of Vienna in 1998. The culture was originally derived from a population collected in poultry litter in Germany and has been maintained in mass culture identical to the other two populations. The populations will be referred to as *Germany*, *Stirling* and *Balmerino* from here on. Population was entered as a random factor in our analyses as these populations are a random sample of all possible *S. berlesei* populations. Mass culture involves maintaining the population of mites in a 50 mL vial with *ad libitum* powdered yeast and wheat-germ at a 3 : 1 ratio, the vial secured with a dense cotton wool plug. These cultures were kept in separate desiccators at 90% humidity buffered by KOH solution (153 g L<sup>-1</sup> H<sub>2</sub>O).

### Isolation of larvae

In order to collect first instar nymphs from the populations, c. 100 mites were transferred from the culture to an 'isolation dish'. These dishes were 100 mm diameter Petri-dishes with 5 mm of solid plaster of Paris in the base. The plaster was made damp with drops of water and powdered yeast lightly sprinkled onto the plaster. Samples of each population were introduced to three different isolation dishes per population. A piece of moist tissue paper was placed over the dish and the lid, with a

50 mm diameter hole cut into it, was replaced. Isolation of first instar larvae were made three days after the samples from the culture were placed on the isolation dish, i.e. isolated larvae were transferred from stock cultures as eggs about to hatch. Isolation of first instar larvae took place on three consecutive days, isolation was done by each of us and from all three isolation plates on each day.

On each of the 3 days, larvae were moved from isolation dishes either into individual vials, or into vials of 10, 20, 30 or 40 larvae. On each of the 3 days of isolating nymphs, for each population, 80 nymphs were placed into individual vials, eight vials of ten nymphs were isolated, four vials of 20 nymphs, three vials of thirty nymphs, two vials of 40 nymphs. Where possible, each of us isolated larvae for each population and density treatment on each replicate day. On the final day of isolation the sample size was reduced because of a lack of available larvae for six vials of 10 (four in *Stirling*) and two vials of 20, 30 and 40. The vials for individually isolated larvae were made of 15 mm long glass tube with a 8 mm diameter. The base of the tube was filled with a mixture of plaster and powdered charcoal, vials contained one ball of *Alinson's* dried yeast, and were closed with a bung of nonabsorbent cotton wool. The vials were kept damp by placing them on a Petri-dish with filter paper dampened in the base. The vials in which the density treatments were reared were the tops of 25 mm diameter 'universal' plastic vials. These had a screw cap in which a 5 mm hole had been drilled in the centre and a cotton wool bung inserted. The base of these vials was of plaster and charcoal, and they were kept moist on damp filter paper. The number of yeast balls available to groups of ten nymphs was 3, groups of 20 was 6, 30 was 9 and 40 was 12. The vials containing each day's replicates of vials were placed into plastic *tupperware* containers with damp filter paper on the base. For each of the three days of isolation, the vials containing the isolated larvae were in one container, and those with densities of 10, 20, 30 and 40 were in a second and third, the placement of the populations and densities was randomized within the containers.

### Weighing tritonymphs and scoring morph ratio

The final instar of the mites' development is termed the tritonymph. Tritonymphs pass through a quiescent phase lasting 8–12 h prior to eclosion as adults. At this readily recognizable stage they are immobile and easily weighed. The vials containing individually isolated larvae were checked every morning and afternoon after 4.5 days of development for larvae in or approaching the quiescent state. Tritonymphs approaching the quiescent stage were further monitored every midday and evening. Quiescent tritonymphs were removed and weighed on a *Sartorius supermicro* balance to 0.0001 mg. Quiescent tritonymphs weight (QTW) is known to be highly repeatable (Radwan

*et al.*, 2002). Individually isolated larvae that eclosed into males were weighed as adults at their stable adult weight, 2 days after eclosion (Radwan *et al.*, 2002).

Males from *Stirling*, that were reared in isolation and weighed as quiescent tritonymphs and as adults, were used to investigate the genetic basis to morph determination of this population. Males were mated with two females and 20 progeny from each female were isolated. The progeny were not weighed for logistic reasons (we were unable to weigh >2000 nymphs), but the sex and male morph of the adult mites was recorded from the fifth day of isolation onwards.

## Results

### Status-dependent expression of the fighter morph

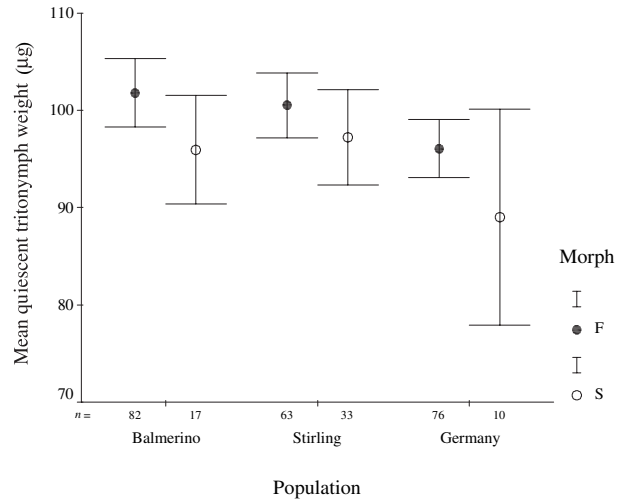
The weight of the individually reared tritonymphs was entered in a two-way analysis of variance, with *morph* as a fixed factor and *population* and *day of isolation* of the larvae as random factors (Table 1). Nonsignificant interactions ( $P > 0.1$ ) were removed from the saturated model. Male QTW differed significantly between populations, but after accounting for this variation, fighters emerged from heavier tritonymphs (Table 1, Fig. 1).

Logistic regression was used to test for variation in the relationship between status and fighter expression in isolated larvae. The logistic regression model with male morph as the dependent variable and tritonymph weight as the covariate was marginally nonsignificant (final model fit  $\chi^2_1 = 3.72$ ,  $P = 0.054$ , Cox and Snell pseudo  $r^2 = 0.03$ ). The addition of *population* to the model significantly increased the model fit, ( $\chi^2_3 = 20.73$ ,  $P < 0.001$ , Cox and Snell pseudo  $r^2 = 0.07$ ) and the likelihood ratios of both tritonymph weight and population were significant ( $-2 LL = 260.19$ , *Tritonymph* likelihood ratio  $\chi^2_1 = 5.46$ ,  $P < 0.05$ ; *Population* likelihood ratio  $\chi^2_2 = 17.01$ ,  $P = 0.000$ ). Further model improvement was sought by including the day on which larvae were isolated from the colony (likelihood ratio  $\chi^2_2 = 2.82$ , n.s.), and development time (likelihood ratio  $\chi^2_1 = 0.940$ , n.s.), these did not however significantly improve the model. Finally, the interaction between QTW and population

**Table 1** General linear model of male quiescent tritonymph weight, confirming that populations differ in tritonymph weight and that morph expression is status-dependent (mean values are shown in Fig. 1).

Source	d.f.	Mean squares	F	P-value
Intercept	1	171 878 027.47*	2018.35	0.000
Population ( <i>r</i> )	2	93 225.92	4.68	0.010
Isolation ( <i>r</i> )	2	44 536.95	2.23	0.109
Morph	1	95 136.03	4.77	0.030
Error	275	19 937.47		

\*0.655 MS(Isolation day) + 0.670 MS(Pop) - 0.325 MS(Error) = 85157.82; d.f. = 3.05.



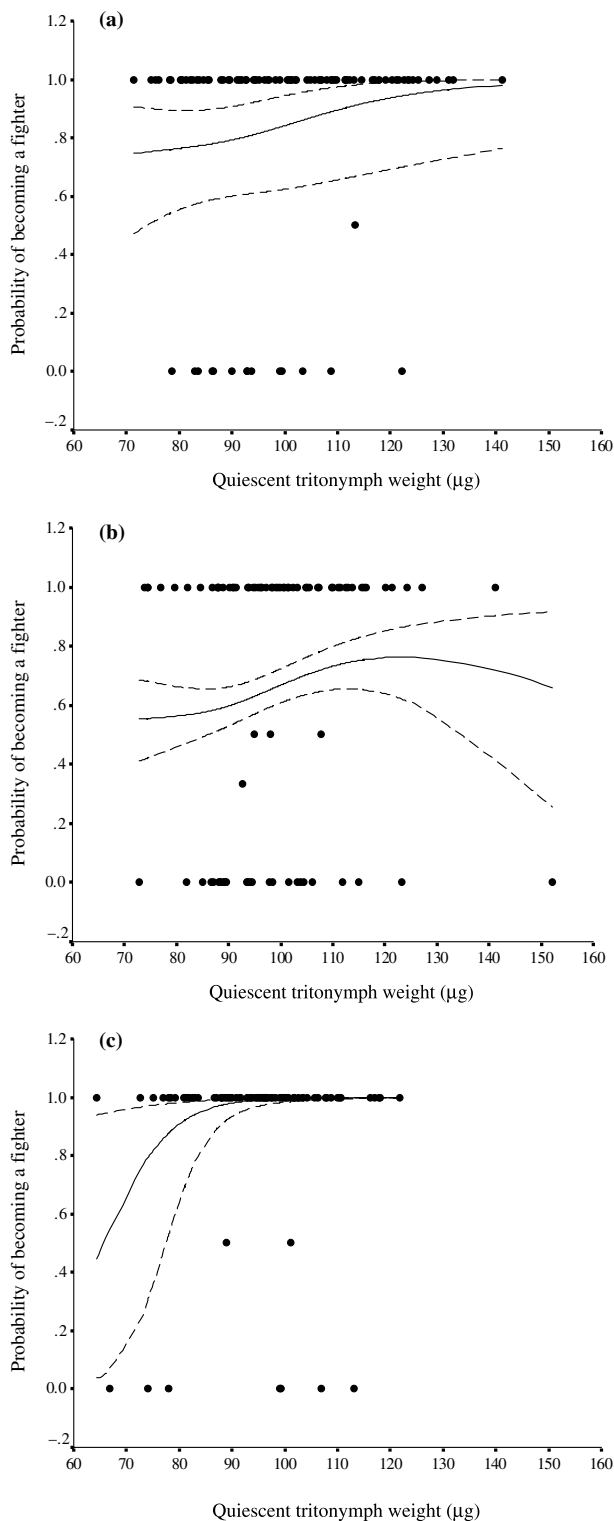
**Fig. 1** Variation in quiescent tritonymph weight and status-dependence of male morphs for the three populations of *Sancassania berlesei*.

was tested against the final model with QTW and population but with no interaction. The interaction did not significantly improve the model fit (interaction  $-2LL = 259.76$ , compared with 260.19,  $\chi^2_1 = 0.40$ , n.s.). These results indicate that the populations differ in the elevation of the relationship between QTW and male morph, but not in slope. The population reaction norm for isolated larvae from each population is shown graphically in Fig. 2, using cubic spline analysis to fit the curve of fighter expression onto the continuous distribution of QTW.

The ratio of fighters to scramblers in the males reared under isolation for the three populations was significantly different [*Balmerino* 82 : 17 (83 : 17% fighters), *Germany* 76 : 10 (88 : 12% fighters), *Stirling* 63 : 34 (78 : 22% fighters)  $\chi^2_2 = 16.54$ ,  $P < 0.001$ ].

### The genetic basis to morph expression in the Stirling population

The morph ratio of offspring from *Stirling* males was analysed with a general linear model (GLM) with sire *morph* as a fixed factor and the QTW and *development time* of the sire as covariates (Table 2). There was a highly significant sire morph effect on the proportion of his male offspring that were fighters. There was an effect of the sire's development time, such that males that developed relatively slowly for their QTW sired more fighter offspring. Finally there was also a significant effect of the sire's QTW on the proportion of offspring becoming fighters (Table 2). Falconer's method for estimating the heritability of liability uses the frequency of the 'affected' group in the population and compares that with the ratio of the affected group in their offspring. If we first consider



**Fig. 2** Cubic splines with SE generated from 500 bootstrap iterations. Splines show the reaction norm of fighter expression in (a) *Balmerino* (b) *Stirling* and (c) *Germany* populations.

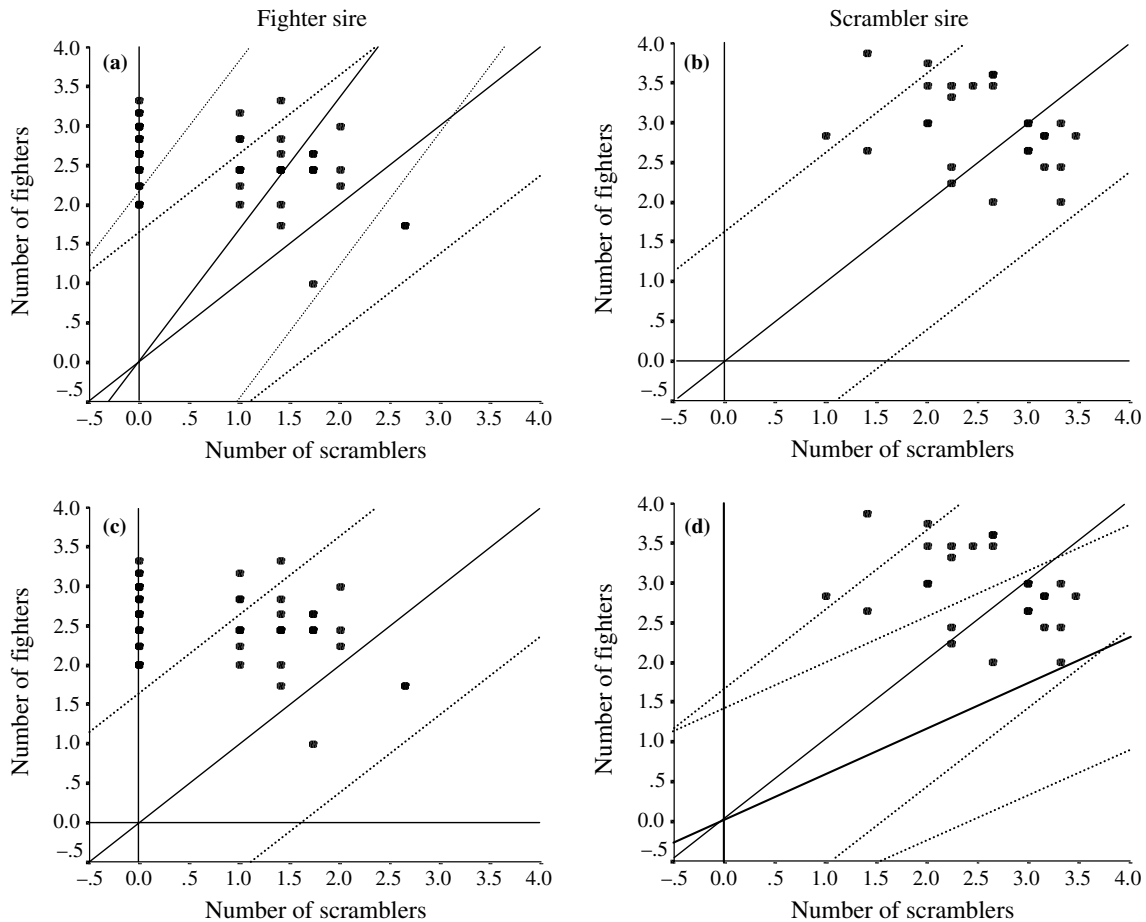
**Table 2** General linear model of the proportion (transformed) of fighters in the offspring of fighter and scambler males of known quiescent tritonymph weight (QTW) and development time. Non-significant interactions were removed.

Source	d.f.	Mean squares	F	P-value
Model	3	0.413	14.03	0.000
Intercept	1	0.069	2.34	0.130
QTW	1	0.127	4.30	0.042
Development time	1	0.148	5.03	0.028
Morph	1	0.710	24.10	0.000
Error	72	0.029		

the affected group to be scamblers which did not produce the fighter phenotype under isolation, these constituted 21.8% of the population and 43.35% of their offspring were scamblers, yielding an estimate for the heritability of 'liability' 0.90. The same calculation for fighters, which represented 78.2% of the isolated population and had 82.4% fighter offspring has a heritability of liability of 0.81.

The morph ratio of offspring from fighter and scambler sires was examined graphically (Fig. 3) for deviation from the 'Mendelian ratios' expected under single-locus inheritance (Mosteller & Tukey, 1949). If the father morph is assumed to be dominant, the expected ratios with unknown male genotype (e.g. FF, Ff) and unknown female genotype (FF, Ff or ff) are 1 : 0, 3 : 1 or 1 : 1, and if recessive (e.g. ff), 1 : 0, 1 : 1 and 0 : 1. The hypothesis that the fighter morph is dominant can be rejected by Fig. 3b in which the offspring of scambler males are shown. Single-locus inheritance does not allow any departures from the expected ratios, and two points lay outside 95% significance zone for 1 : 1 ratio. Moreover in Fig. 3b there are no offspring ratios that were 1 : 0 or 0 : 1 fighters to scamblers, whereas the proportion of homozygotes inferred from the population proportion of fighters was such that only 50% families were expected to contain mixed morphs whereas they all did [the proportion of fighters in the population = 0.78, hence proportion of scambler homozygotes = 0.22, and fighter homozygotes =  $(1 - 0.22^{1/2})^2 = 0.28$ , the proportion of families expected to be homozygous = 0.22 + 0.28 = 0.50]. The excess of families with both male morphs present deviates significantly from expected ( $\chi^2_1 = 13.5$   $P < 0.001$ ). Similarly the hypothesis that scamblers are dominant can be rejected because the offspring ratio cannot be 1 : 0 when the sire is a fighter (Fig. 3c), and several ratios are outside 95% significance zones.

A GLM with arcsine transformed sex ratio as the dependent variable, arcsine *morph ratio* and *mortality* as covariates and father's *morph* as a factor was used to test for any evidence of morph interacting with genes that may be responsible for determining sex. There was no evidence for a sex ratio bias in the offspring of fighter ( $0.57 \pm 0.14$ ) or scambler males ( $0.55 \pm 0.19$ ,  $F_{1,73} = 0.07$ , n.s.), and there was no significant relationship



**Fig. 3** Offspring numbers (transformed to square roots) from fighter (a, c) and scambler (b, d) sires. The expected offspring male morph ratios when a given morph is assumed to be dominant, i.e. a male can be either a homozygote or a heterozygote are 1 : 0, 3 : 1 or 1 : 1 (panels a and d), and if the morphs is recessive, and therefore has to be homozygous, 1 : 0, 1 : 1 and 0 : 1 (panel b and c). Solid lines show expected ratios and dashed lines the limits of two tailed significance (Mosteller & Tukey, 1949), 1 : 0 and 0 : 1 are not expected to have error.

between sex ratio and morph ratio ( $F_{1,73} = 3.29$ ,  $P = 0.07$ ), although the trend was for increased numbers of fighters to be associated with the increased numbers of females. A *post hoc* test showed that this trend was not because of differential mortality ( $F_{1,73} = 0.64$ , n.s.).

### Population variation in the effect of density on morph expression

Increasing colony density is known to suppress the production of the fighter morph (Radwan *et al.*, 2002). To examine the variation between populations in the suppressive effects of density on fighter expression we performed a two way ANOVA. The independent variable was the *percentage of fighter males* (in radians) per vial or per isolation day for individually reared larvae, the fixed factor was *density*, with *population* and *day of isolation* as random factors. There were no significant interactions and all but one was removed from the model. Table 3

shows the fixed factors and the interaction between population and density as this was of specific interest. There was a significant effect of density in decreasing the

**Table 3** General linear model of the effect of increasing density on the proportion of fighters (transformed to radians), and the differences between the populations in the effect of density.

Source	d.f.	Mean squares	F	P-value
Intercept	1	43.47*	11.62	0.072
Population ( <i>r</i> )	2	3.70†	46.06	0.000
Isolation ( <i>r</i> )	2	0.222‡	2.35	0.100
Density	4	0.805§	8.51	0.000
Pop × Density	8	0.0756‡	0.80	0.605
Error	128	0.0946		

\* $0.674 \text{ MS}(\text{Day}) + 0.987 \text{ MS}(\text{Pop}) - 0.661 \text{ MS}(\text{Error}) = 3.74$ ; d.f. = 2.1.

† $0.749 \text{ MS}(\text{Density} \times \text{Pop}) + 0.251 \text{ MS}(\text{Error}) = 0.08$ ; d.f. = 15.9.

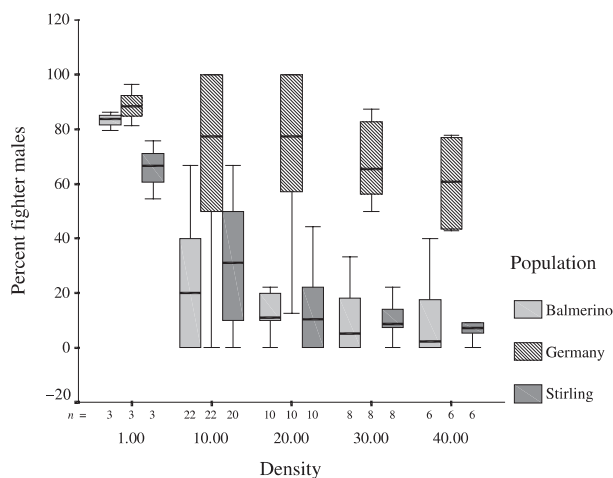
‡MS(Error).

§ $0.998 \text{ MS}(\text{Density} \times \text{Pop}) + 0.002 \text{ MS}(\text{Error}) = 0.076$ ; d.f. = 8.

proportion of fighters emerging in the vials (Fig. 4). The populations differed significantly in the degree to which fighter morph was suppressed, *Germany* maintaining the highest level of expression across the range of densities (Fig. 4). However the rate at which suppression increased with density was not significantly different between populations, indicated by the nonsignificant interaction (Table 3). The decline in fighter frequency appears to be most divergent over the increment in density from isolation to 10 per vial. *Post hoc* analysis of the frequency of fighters derived from isolated vials compared with groups of 10 showed no significant interaction under the same model as the previous analysis (population  $\times$  density,  $F_{2,65} = 1.934$ ,  $P = 0.15$ ).

### Population variation in the somatic costs of fighter morph expression

Previous data (Radwan *et al.*, 2002) has shown that in *Germany* the production of the fighter phenotype incurs a cost to the male mite in terms of adult size and weight. We examined the generality of this cost across the three populations studied here. Adult weight was used as the dependent variable in a GLM, with *morph* as fixed factor and *population* and *day of isolation* as random factors and *QTW* as a covariate. Nonsignificant interaction terms were removed sequentially from the model to leave a reduced model containing only two interactions (Table 4). The GLM reveals a significant interaction between the day of isolation and the relation between *QTW* and adult weight and also that the different populations were influenced differently by the day of isolation. This makes the interpretation of population effects difficult in this analysis, but leaves the analyses of the difference between the morphs open to interpretation. At



**Fig. 4** Medians (bar), quartiles (box) and range (whiskers) of percent fighters produced by *Balmerino*, *Stirling* and *Germany* populations with increasing density. Statistics are shown in Table 3.

**Table 4** Analysis of covariance between adult weight and tritonymph weight including male morph as a fixed factor and population and the day of isolation as random factors.

Source	d.f.	Mean squares	F	P-value
	1	46 951.54*	2.57	0.250
Population ( <i>r</i> )	2	10 376.91†	0.78	0.515
Morph	1	83 804.73‡	16.14	0.000
Isolation ( <i>r</i> )	2	18 498.13§	3.45	0.033
Tritonymph	1	6 596 428.76‡	1270.52	0.000
Population $\times$ Isolation	4	13 479.22‡	2.60	0.037
Isolation $\times$ tritonymph	2	16 451.39‡	3.17	0.044
Error	268	5 191.91		

\*0.989 MS(Isolation) + 1.139E-02 MS(Error) = 18 288.18; d.f. = 2.0.

†0.970 MS(Pop  $\times$  Isolation) + 2.954E-02 MS(Error) = 13234.41; d.f. = 4.1.

‡MS (Error).

§2.004E-02 MS(Pop  $\times$  Isolation) + 0.980 MS(Error) = 537.98; d.f. = 250.

**Table 5** Analysis of covariance between adult weight in fighters and their weight as a tritonymph, population and day of isolation are both random factors.

Source	d.f.	Mean squares	F	P-value
Intercept	1	36 079.10*	6.65	0.011
Population	2	19 566.35	3.71	0.026
Isolation ( <i>r</i> )	2	12 874.52	2.44	0.090
Tritonymph weight	1	5 297 856.22	1004.24	0.000
Error	215	5 275.48		

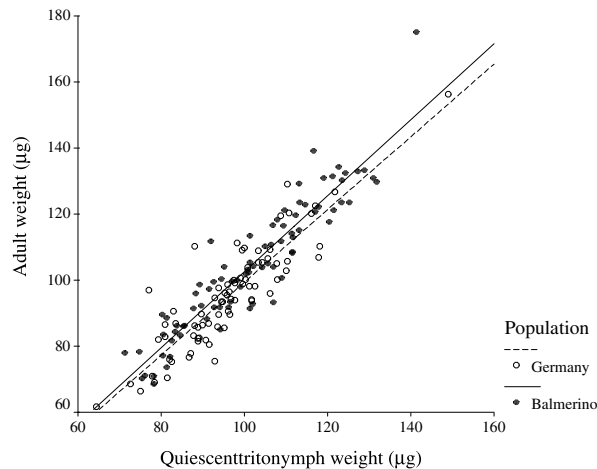
\*0.020 MS(Pop) + 0.020 MS(Isoloday) + 0.960 MS(Error).

the mean *QTW* of 0.9862 mg adult fighters weighed  $0.9934 \pm 0.0049$ , while scramblers were significantly heavier at  $1.0378 \pm 0.0097$  mg.

The analysis of the fighter morph alone allows population variation in the cost of producing the fighter phenotype to be examined. Table 5 shows an analysis of covariance in which only fighters are considered. The dependent variable is adult weight and the covariate is tritonymph weight. There were no significant population-by-*QTW* interactions indicating that the slopes of adult weight on *QTW* were parallel, there was significant variation between populations in the elevation of the slopes. These differences arising predominantly between *Germany* and *Balmerino* populations [least significant difference (LSD),  $P < 0.01$ ] rather than *Germany* and *Stirling* (LSD, n.s.) or *Balmerino* and *Stirling* (LSD, n.s.). The difference in elevation of the adult weight on tritonymph weight for *Balmerino* and *Germany* only, is shown in Fig. 5.

## Discussion

The conditional ESS model, with status-dependent alternative male tactics, is the most commonly inferred model



**Fig. 5** Population variation in the somatic cost of producing the fighter phenotype; *Balmerino* males eclosed as heavier fighters than *Germany* males of an equivalent quiescent tritonymph weight.

for the evolution of alternative reproductive tactics (Gross, 1996). Although an association between status and dimorphic morphology or behaviour has been widely reported (Gross, 1996), Shuster & Wade (2003) suggest that the fit of the status-dependent model is more apparent than real. We have tested hypotheses central to the status-dependent ESS model, in three populations. We found substantial population variation and no patterns that are incompatible with the status-dependent ESS model.

### Choice of male morphology

The status-dependent ESS predicts that individuals make a decision with regard to the tactic that they adopt, and that they adopt the tactic from which they will derive the highest fitness return (Gross, 1996). We have not addressed the fitness benefits of the alternative tactics here, although it is known that at low colony sizes fighter males are likely to have an advantage over scramblers because they are able to kill all of their rival males and dominate a group of females (Radwan, 1993). Thus, density is likely to change the point of intersection between fitness functions for fighters and scramblers. We expected that males will make adaptive decisions about their morph in response to the relevant cue, which in these mites is a chemical from dense colonies on which fighter expression is partially dependent (Woodring, 1969; Timms *et al.*, 1980; Radwan, 1995). Our results show that across all three populations there was a decline in fighter male frequency with the increase in the density at which the mites were reared. These data demonstrate that male mites that would typically adopt one tactic at one density can adopt the alternative tactic at another. Previous work has shown that there is no decrease in status under conditions where the fighter morph is

suppressed with colony pheromones (Radwan *et al.*, 2002) however a decline in QTW has been shown with real increases in density (Radwan, 1992). The suppression of the fighter morph with increasing density is therefore likely to be due both to a shift in switch point (Radwan, 1992) and a shift in the distribution of QTW (Radwan *et al.*, 2002). Shuster & Wade (2003, p. 401) have suggested that observations of status-dependent tactics require that 'only part of the population responds to environmental cues'. Our data can be used to estimate the proportion of the populations that are responding to environmental cues. For *Balmerino* for example, fighter phenotypes were expressed by 83% of the population under conditions of isolation, and 10% of individuals at a density of 40. Depending which morph is considered to be responding to the environment, the part not responding is either 10% (if scramblers are responding) or 17% (if fighters are responding). Evidently the vast majority of the population are responding to the environment. *Germany* has higher fighter expression than *Balmerino*, and was suppressed to a lesser extent by increasing pheromone concentration, and yet fighters in this population can still be suppressed completely in a large colony (J. Radwan & J.L. Tomkins, personal observation).

### Status-dependence

The dimorphic reaction norms of species such as dung beetles or earwigs have been characterized by the relation of the dimorphic trait length (horns or forceps) plotted against a measure of status, usually a linear measure of body size (Eberhard & Gutierrez, 1991; Emlen, 1994; Simmons *et al.*, 1999; Tomkins, 1999; Tomkins & Simmons, 2000; Forslund, 2003). We characterized the dichotomous variation in male morph in *S. berlessei* with a cubic spline that depicts the change in the probability of an individual being a fighter or a scrambler over the range of QTW represented in the population. The GLM (Table 1) and the logistic regression analysis confirmed that there was status-dependence across the three populations. Morph expression consistent with the status-dependence model has previously been reported in this species for *Germany* (Radwan *et al.*, 2002), but not for *Stirling* or *Balmerino*.

### Polyphenism and the heritability of liability

The status-dependence observed in isolated larvae is likely to be relatively weak compared with larvae that are reared in groups large enough to cause 50% to become scramblers (as in Radwan *et al.*, 2002). Weak status-dependence in isolated larvae occurs because one of the factors controlling the proportion of fighters is the concentration of chemicals that emanate from dense colonies. In isolated larvae, the expectation is therefore for most males to produce the fighter phenotype – even

males of the lowest status. The advantage of examining the cubic splines for isolated larvae is to reveal what proportion, and of what status, are the males that do not produce the fighter phenotype, even when conditions are ideal to do so. The high elevation of the *Germany* spline (Fig. 2c) indicates that most males are capable of fighter expression, a result consistent with the notion that males are phenotypically plastic with respect to the ability to express the fighter phenotype. In contrast, the data for *Stirling* suggest that many males, even of high status are incapable of fighter production. Rather than a classical reaction norm of the response of an individual genotype expressed over a range of environments (Schlichting & Pigliucci, 1998), the splines in Fig. 2 show the population response to increasing status. This means that the cubic splines reveal only a summary of the responses of individuals within each population. What is evident is that under isolation *Stirling* has a much lower expression of the fighter phenotype than *Germany* or *Balmerino*. This difference between populations in the elevation of the population reaction norm may indicate one of two situations. First, there may be status-dependent morph expression, but with more genetic variance in the switch point i.e. some males become fighters at a low status, whereas others become fighters only at very high status. Note that this is still consistent with polyphenism as the strategy determining alternative tactics, as all males can still become either a scrambler or a fighter for some value of status. Alternatively, the low elevation of the reaction norm may indicate that some individuals have lost the ability to produce the fighter phenotype regardless of status (e.g. Lively *et al.*, 2000): a situation where the population is no longer genetically monomorphic for the strategy.

Lively *et al.* (2000) attempted to distinguish between these mechanisms to explain the lack of induction of defence in a barnacle *Chthamalus anisopoma* by a predator. They did so by increasing the exposure of barnacles to predators. They observed an asymptote in the dose-response curve to increased exposure, which they interpreted in support of the 'polymorphism' model. However, in *S. berlesii* such a test was not possible as we observed complete suppression of fighters in dense colonies, rather an explanation was needed for the nonexpression of fighters under the absence of the cue. Thus, in an attempt to address these two possibilities for the unusually low levels of fighter expression, even among some high status males in *Stirling*, we examined the proportion of fighters produced among their offspring. The analysis of offspring morph ratios shows that there is a significant effect of the sire's QTW on the proportion of his offspring that were fighters. This is consistent only with the liability model for the evolution of threshold characters (Falconer & Mackay, 1996; Roff, 1997) in which an underlying character, 'liability', is normally distributed and heritable and a fixed switch point determines morph expression. Our estimate of the

heritability of liability approached 1. The heritability of liability model is based on genetic variation in liability and does not assume any significant genetic variation in thresholds. The strong morph effect in the model (Table 2) makes it clear that the high heritability of liability is not solely because of the heritability of QTW. Hence the high heritability may be due to the extreme variation between individuals in switch point, inflating the estimate of the heritability of liability (see below). In a male dimorphism with status-dependent tactics, liability should be closely related to status. We found a significant effect of sire QTW on morph suggesting that indeed QTW shows some characteristics of liability. This sire QTW effect on morph suggests that the offspring from heavy sires were more likely to fall on the fighter side of the threshold(s) than were the sons of light sires. Despite its importance to the understanding of the evolution of alternative reproductive tactics, the heritability of liability has only rarely been documented as specifically contributing to the inheritance of male morphs (Gross & Repka, 1998; see also Hazel *et al.*, 1990). The heritability of body size (which may contribute to the liability) has been estimated for various male dimorphic species; although for the most part, these estimates have been not significantly different from zero (Emlen, 1994, 1996; Moczek & Emlen, 1999; Hunt & Simmons, 2002; Kurdziel & Knowles, 2002) or else low (Tomkins & Simmons, 1999; Kotiaho *et al.*, 2003) and influenced by indirect-genetic paternal and maternal effects (Kotiaho *et al.*, 2003).

The heritability of 'liability' is an integral feature of the inheritance of threshold traits (Falconer & Mackay, 1996; Roff, 1997). Here however we found that after controlling for sire QTW there was still a highly significant effect of sire morph on the proportion of fighters among a male's offspring. The latter observation is compatible with the loss of ability to produce the fighter morph in some males, but also with there being considerable genetic variation in the switch point. For example, the offspring of scrambler male, whose switch point lies far to the right of the status distribution, will most likely be scamblers, even controlling for their father's QTW. Similarly, males whose switch point is far to the left will have mostly fighter offspring and be fighters themselves as most of the status distribution will be to the right of the switch point. Whether the loss of fighter potency is a consequence of switch points beyond the range of status, or an absolute disconnection from status, is an intriguing question. The graphical test for the presence of Mendelian ratios (Fig. 3) in the inheritance of male morph does not reveal a pattern consistent with single-locus inheritance. Hence we can rule out the possibility that the loss of fighter potency in this population is solely due to a single-locus determining male morph. With this experiment we cannot rule out the possibility that threshold expression is only a property of heterozygous individuals. This would require further testing.

Our results can be ascribed to extreme genetic variation in reaction norms within the population and it does not seem sensible to extrapolate to single-locus inheritance and an absolute loss of fighter potency. Strong sire-morph effects found for *Stirling* are in contrast to the findings of Radwan (1995) who did not find such an effect in a population obtained from poultry litter in Wołowice, Poland. The Wołowice population was more extreme than *Germany* in producing 97.2% of fighters under isolation. This indicates that populations may differ not only in mean QTW, in the threshold of fighter expression and/or in the threshold of sensitivity to pheromones (this study), but also in the heritability of male morph. Although the number of sires in Radwan's (1995) study ( $n = 21$ ) was smaller than in the present study, there was no evidence for an effect ( $P = 0.745$ ) despite moderate power (0.59) to detect effects of the magnitude found in *Stirling*. Strong sire-morph effects on offspring morph ratio were detected in Radwan (1995) in another acarid *Rhizoglyphus robini*. Heritabilities of liability estimated for *R. robini* exceeded unity, questioning the adequacy of the liability model in that species (Radwan, 1995), but did not fit any simple, single-locus Mendelian segregation model, a finding similar to that reported here for *Stirling*. Unlike *Stirling*, however, morph ratio in *R. robini* was not affected by colony density, so the strong inheritance of male morph could not be due to intra-population variation in the threshold of response to colony chemicals. Another estimate obtained from different population of *R. robini* selected on alternative morphs yielded lower estimates of morph heritability (range: 0.3–0.8), which would be consistent with liability models (Radwan, 2003). The data for *R. robini* therefore also indicate substantial interpopulation variation in heritabilities of male morph. Status-dependence of male morphs has not yet been tested in *R. robini*, but male morph has been manipulated through nutritional stress (Radwan, 1995).

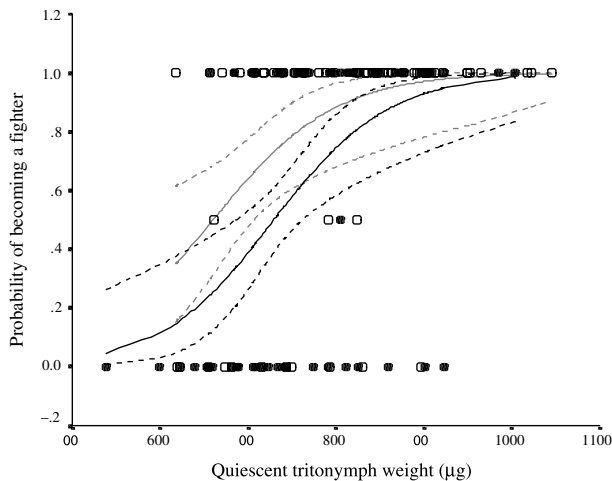
### Evolutionary divergence in ESS switch points between populations

Status is a measure of competitive ability that is measured relative to other members of the population, hence status is a population specific parameter (Gross, 1996). The status-dependent ESS model therefore predicts that switch points will evolve independently from any divergence in the mean size of individuals between populations. It is expected that demographic, behavioural or ecological divergence between populations will determine the switch point in each population. We used tritonymph weight as our index of status within each population as it is during the tritonymphal stage that morph is determined (Woodring, 1969). Mean QTW also provides a measure of the divergence in size between populations. We found a significant difference between the three populations in the proportions of fighter males

that emerged from isolated larvae, and as outlined above, this variation suggests that some parameter of morph expression differs between the populations. In accordance with this notion of independent evolution of switch point and body size, the populations differed significantly in status (QTW). Importantly, mean population QTW was independent of the proportion of fighters produced: *Germany* males were the lightest at the QT stage, but produced the highest proportion of fighters from both isolated and group reared larvae. Two previous studies of male dimorphisms have revealed similar divergence in switch point independent of status in species reared in a common garden experiment. Moczek *et al.* (2002) found that the dung beetle *Onthophagus taurus* has divergent switch points between exotic populations in Western Australia and North Carolina in the USA. On a finer scale, Tomkins (1999) showed that island populations of earwigs separated by <2.5 km of sea had divergent switch points; here too the smaller bodied population had a higher frequency of the major 'macrolabic' males. These between population differences undoubtedly reflect selection acting on the ESS switch point. Direct evidence for genetic variance in switch points in male dimorphic traits in other species comes only from selection experiments conducted in the dung beetle *O. acuminatus* (Emlen, 1996).

### Population variation in suppression

*Sancassania berlesei* differs from many insect dimorphisms in that status-dependent expression is affected by the concentration of colony chemicals. The data for isolated males reveal the variation within and between the populations in status (QTW), and the level of fighter expression in relation to status. Our data also show that the populations differ significantly in their response to increasing density. In common with the isolated males, group reared *Germany* produced the highest proportion of fighters, and maintained high fighter expression across the range of densities. In a previous study of *Germany* we reared two treatment groups at different pheromone pressures (Radwan *et al.*, 2002). In that study we failed to demonstrate that the change in morph frequency was because of a shift in the lateral position of the switch point (Radwan *et al.*, 2002). However, we did not use logistic regression to address the question. Re-analysis of that data confirms that the decrease in fighter frequency was because of a shift in the switch point towards higher values of tritonymphal weight (tritonymph weight, likelihood ratio  $\chi^2 = 30.34$ , d.f. = 1,  $P < 0.001$ , pheromone treatment, likelihood ratio  $\chi^2 = 4.822$ , d.f. = 1,  $P = 0.028$ , replicate, likelihood ratio  $\chi^2 = 5.595$ , d.f. = 1,  $P = 0.018$ ; Fig. 6). In the current study the shifts in morph ratio with increasing rearing density most likely reflect a similar process: the switch point shifts to the right of the status distribution increasing the proportion of scambblers. Under the status-dependent ESS a



**Fig. 6** Cubic spline depicting the reaction norm of fighter expression in two treatments of Germany, one (left hand spline open circles) reared with low levels of colony pheromone and the other (right hand spline closed circles) reared at high levels of colony pheromone. Data are from re-analysis of Radwan (2001) but the lateral shift in reaction norm was not detected in that publication.

decrease in fighter frequency with increasing density suggests a change in fitness functions of the alternative tactics, as documented by Radwan (1993) for another population of *S. berlessei*. The divergence between populations can be viewed as reflecting the different rates at which the fitness functions change in each population as population density increases.

### Population variation in the costs of the fighter phenotype

We investigated the possibility that the cost of producing the fighter phenotype might be a contributing factor in the variation between populations in the rate of fighter suppression with increasing colony density. The cost of producing the fighter phenotype is manifested as a reduction in final adult size and weight (Table 4, Fig. 5 and Radwan *et al.*, 2002). There is a positive covariance between the size of fighters and their longevity and mating success (L. Michalczyk, N.R. LeBas, J. Radwan & J.L. Tomkins, unpublished data), hence the reduction in size associated with becoming a fighter may have significant fitness consequences. Contrary however to the expectation that *Germany* males would suffer a lower cost of fighter expression, and so have a greater frequency of fighters in the population, the costs of fighter expression in terms of final weight for equal QTW were significantly greater for *Germany* than for *Balmerino*. The relatively high proportion of fighter males in *Germany* cannot therefore be explained by the morphological cost of fighter expression. It remains to be tested whether there are behavioural differences between the populations, in particular

whether fighters in *Germany* are more aggressive than fighters in the other populations.

### Conclusions

We have found support for key aspects of the status-dependent ESS across three populations of *S. berlessei*. We also found considerable variation between populations in the dynamics of morph expression. This variation is consistent with our predictions about the evolution of status-dependent male dimorphisms. Namely, that population variation in ESS switch point will give rise to the independent evolution of status and morph ratio, and that the heritability of QTW underlies at least some of the inheritance of male morph in some populations of this species. In addition, we have revealed another pattern of morph inheritance that is consistent with high levels of genetic variation for the switch point in one of the populations under study. Whether the fitness functions of the alternative tactics cross at the phenotypic switch point as predicted, remains a critical test of the status-dependent ESS in this species and is currently under test.

### Acknowledgments

We thank Barry Sinervo for his comments on an earlier draft. The Royal Society funded a joint project grant to JLT and JR, JLT is a BBSRC Research Fellow and NRL is a NERC Research Fellow.

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Received 17 November 2003; accepted 27 January 2004