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PHENOTYPIC ASSORTMENT MEDIATES THE EFFECT OF SOCIAL SELECTION IN A WILD BEETLE POPULATION

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Social interactions often have major fitness consequences, but little is known about how specific interacting phenotypes affect the strength of natural selection. Social influences on the evolutionary process can be assessed using a multilevel selection approach that partitions the effects of social partner phenotypes on fitness (referred to as social or group selection) from those of the traits of a focal individual (nonsocial or individual selection). To quantify the contribution of social selection to total selection affecting a trait, the patterns of phenotypic association among interactants must also be considered. We estimated selection gradients on male body size in a wild population of forked fungus beetles (*Bolitotherus cornutus*). We detected positive nonsocial selection and negative social selection on body size operating through differences in copulation success, indicating that large males with small social partners had highest fitness. In addition, we found that, in low-density demes, the phenotypes of focal individuals were negatively correlated with those of their social partners. This pattern reversed the negative effect of group selection on body size and led to stronger positive selection for body size. Our results demonstrate multilevel selection in nature and stress the importance of considering social selection whenever conspecific interactions occur nonrandomly.

KEY WORDS: ***Bolitotherus cornutus*, contextual analysis, interacting phenotypes, multilevel selection, sexual selection, social evolution.**

Social interactions among conspecifics influence the evolutionary process because they typically generate variance in fitness (West-Eberhard 1979; Wolf et al. 1998; 1999; Bijma et al. 2007; McGlothlin et al. 2010). Some of these fitness effects result from the mere fact of group membership, whereas others depend upon the nature and outcome of interactions ranging from agonistic behavior to cooperation. Many efforts to understand the evolutionary importance of social interactions have focused on the fitness consequences of social behaviors and associations (Wilson 1975b; McGlothlin et al. 2010; Szekely et al. 2010; Westneat and Fox 2010), but comparatively less attention has been paid to how specific phenotypes involved in social interactions influence the targets and strength of selection (Wolf et al. 1999; Wilson and Wilson 2007; McGlothlin et al. 2010). If we are to understand how social behavior drives phenotypic evolution, we must be able to connect social interactions and social environments to their effects on specific characters in the context of multivariate evolution.

Interactions among conspecifics often involve traits such as weapons used in combat, which may vary in size and effectiveness, or dynamic behavioral signals used in communication, which may vary in rate and intensity (Andersson 1994; Shuster and Wade 2003; Andersson and Simmons 2006; ten Cate and Rowe 2007; Alonzo 2010). Trait-based interactions generate the potential for phenotypes in one individual (or group of individuals) to influence the fitness of another. Such socially mediated fitness effects, known as social selection (as well as group or neighbor-mediated selection), can profoundly influence the covariance between phenotype and fitness at the population level and may account for a substantial component of multivariate evolutionary change (Queller 1992; Wolf et al. 1999; Okasha 2006; Bijma and Wade 2008; McGlothlin et al. 2010).

Most studies that investigate phenotypic selection in natural populations have not incorporated the effects of conspecific interactions, and thereby implicitly assume that the traits of social partners have no net effect on expected fitness. Social selection can be detected by taking a multilevel selection approach that expands regression models to include not only traits of a focal individual but also those of a social partner or context. This method is mathematically similar to contextual analysis (Heisler and Damuth 1987; Goodnight et al. 1992), which is often used to partition individual- and group-level components of selection (Stevens et al. 1995; Goodnight and Stevens 1997; Aspi et al. 2003; Donohue 2004; Weinig et al. 2007; Eldakar et al. 2010). However, the measurement of social selection differs slightly from traditional contextual analysis in one important respect—the method of calculating the group phenotype. Typically in contextual analysis, the group selection component measures the effect of the group mean, which includes the phenotype of the focal individual. Social selection includes only the phenotypes of other individuals with which the focal interacts (Wolf et al. 1999; Bijma et al. 2007; Bijma and Wade 2008; McGlothlin et al. 2010). Social selection is thus identical to the neighbor-mediated model of multilevel selection discussed by Nunney (1985) and Okasha (2006). The social selection partitioning has advantages for certain applications. For example, when group membership is fluid or the frequency of interactions differs among group members, the social selection approach allows the group phenotype to be calculated as a weighted mean. In addition, the social selection partitioning leads to a natural interpretation in Hamilton's rule and predictive equations for evolutionary response (Wolf et al. 1999; Bijma et al. 2007; Bijma and Wade 2008; McGlothlin et al. 2010). Despite these differences, the nonsocial and social selection gradients are interpretable as within-group and between-group gradients, respectively (Wilson 1975a; Goodnight et al. 1992; Stevens et al. 1995; Goodnight and Stevens 1997; Wilson and Wilson 2007; Eldakar et al. 2010).

Although all formulations of multilevel selection parse the fitness effects of individuals and groups, the social selection approach further considers the patterns of phenotypic assortment that translate social fitness effects into total selection on specific traits. Wolf et al. (1999) formally partitioned the total effect of selection (s) on a phenotype as

$$s = P\beta_N + C^{ij'}\beta_s. \tag{1}$$

Here, the nonsocial (or individual) selection gradient (β_N) is estimable as the partial regression of relative fitness on the trait of a focal individual (hereafter "focal phenotype") and is thus analogous to the selection gradient typically measured using the method of Lande and Arnold (1983). The effect of nonsocial selection is scaled by the phenotypic variance of the trait expressed in focal individuals (P). The social (or group) selection gradient (β_S) is the partial regression of the relative fitness of a focal individual on a phenotype expressed in one or more social partners (hereafter "social phenotype"). The interactant covariance ($C^{ij'}$) describes the assortment of social and focal phenotypes, translating social fitness effects into phenotypic selection at the population scale. $C^{ij'}$ is calculated as the covariance between focal individuals' phenotypic values (z_i) and the phenotypic values of each focal individual's social partner ($z_j{}'$; here and elsewhere, primes are used to denote variables of social partners). When there are multiple social partners the social phenotype can be defined as the mean phenotype of all social partners ($\bar{z}_j{}'$). Although we describe a univariate case here, the above formulations can be easily adapted to multivariate analysis (Wolf et al. 1999; McGlothlin et al. 2010).

The formulation above reveals that the impact of social selection on phenotypic evolution depends on two separable phenomena. The social selection gradient, β_S, describes the fitness consequences of interacting with social partners with particular phenotypes, as in other multilevel selection analyses. The interactant covariance ($C^{ij'}$) measures the phenotypic assortment of individuals and translates the effects of β_S onto particular traits at the population level. We expect nonzero values of $C^{ij'}$ to be common in nature as such covariance can be generated by phenomena that cause individuals with specific trait values to associate nonrandomly. These may include spatial or temporal segregation of phenotypes due to population structure, relatedness, or trait preferences (Wolf et al. 1999; Formica and Tuttle 2009; McGlothlin et al. 2010; Stamps and Groothuis 2010). For behavior and other plastic traits, nonrandom interactions may arise when interacting individuals influence one another's phenotypic expression through behavioral modification or indirect genetic effects.

To examine whether social interactions generate the covariances and fitness consequences necessary for social selection to effect total selection, we assessed copulation success and survival in a natural population of forked fungus beetles, *Bolitotherus cornutus*. This system is ideal for measuring social selection because *B. cornutus* live in spatially subdivided populations and perform all social and reproductive behaviors in the open. In addition, males conspicuously vary with respect to thoracic horn length, a trait that has been previously demonstrated to be under strong sexual selection (Conner 1988). Because sexual selection involves interactions among conspecifics, sexually selected traits are likely to be subject to social selection. We first asked whether phenotypes of social groups varied among focal individuals because this variance is a necessary condition for social selection to occur. Next, we investigated whether the social phenotype covaried with focal individual phenotypic values (C^{ij}) and whether it covaried with the copulation success or survival of focal individuals (β_S). Finally, we combined these measurements to estimate the contribution of social selection to total phenotypic selection (s).

Methods

STUDY SYSTEM

The entire life cycle of *B. cornutus* (Coleoptera: Tenebrionidae) occurs on polypore shelf fungi, primarily of the genera *Ganoderma* and *Fomes*, and associated dead trees (Brown and Bartalon 1986; Conner 1988). *Bolitotherus cornutus* adults feed on the surface of the fungus and the larvae feed on and develop inside of the fungal brackets (Liles 1956; Pace 1967). Reproductively significant behaviors such as mating and egg-laying take place on bracket surfaces (Conner 1988). Individuals can live for several years, overwintering as either larvae or adults in the fungal brackets and under the bark of associated logs (Pace 1967). Males (but not females) are characterized by thoracic and clypeal horns, which are highly variable in length and positively allometric with body size (Fig 1A; Brown and Bartalon 1986). Both the sets of horns are used in fights between males (Brown and Bartalon 1986; Conner 1988, 1989a), often to pry a courting rival from atop a female (Brown and Bartalon 1986; Fig. 1B.), but do not appear to be used by females in mate choice (Brown et al. 1985). Horn length is continuously distributed, exhibiting no conspicuous dimorphism (Brown and Siegfried 1983) and does not change during adulthood.

Male *B. cornutus* have a stereotyped series of reproductive behaviors that allowed us to accurately score successful copulations during behavioral scans. During courtship, males mount females head to abdomen, then during copulation the males reverse direction and remain facing head to head during the mate guarding stage (Brown and Bartalon 1986). This guarding phase typically lasts from half an hour to several hours and has been demonstrated to be a reliable indicator of insemination success (Conner 1988, 1989b).

Conner (1988, 1989a) found that males with longer thoracic horns gained more inseminations (i.e., horn length was under positive directional sexual selection) in wild populations of *B. cornutus*; this relationship was significantly stronger in populations with low density than in those with high densities. Conner's selection analyses used traditional Lande-Arnold methods, implicitly assuming that social selection did not contribute to total selection (that is, $C^{ij'}\beta_S = 0$).

FIELD METHODS

All work was conducted in the Jungle Trail metapopulation of *B. cornutus* near Mountain Lake Biological Station (37°22′3.63″N, 80°32′2.74″W) in the Appalachian Mountains of southwestern Virginia. We define a single deme as consisting of the beetles inhabiting a single dead tree and its associated fungal brackets (sensu Whitlock 1994). For this study, we chose six demes that

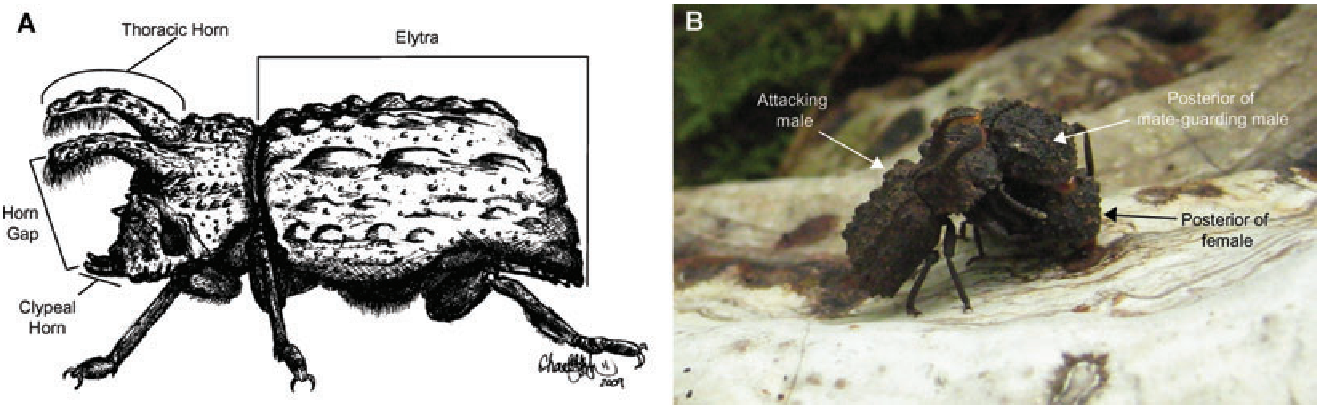

Figure 1. **(A) Line drawing of a male forked fungus beetle illustrating the phenotypes measured for this analysis. The pronotum width was also measured but is not visible from this perspective. Drawing by C. L. Glenn. (B) A large male *B. cornutus* uses his clypeal horns in an attempt to dislodge a smaller male who is mate-guarding a female on the surface of a fungal bracket.**

were located within a 0.5-km radius and contained a large number of resident beetles and fungal brackets with live growth. The only fungus with live growth present at the six sampled sites was *Ganoderma applanatum*.

The surface of each log was gridded using a Mercator-like coordinate system. Nails, spaced 10 cm apart, were labeled with the appropriate coordinates and driven into the center of each grid square. The location and size of all fungal brackets was noted, and all fungal brackets were given a unique ID. This gridding system allowed us to map the location of every beetle present on the surface of the log and brackets with an accuracy of 10 cm (Formica et al. 2010).

PHENOTYPING AND BEHAVIORAL OBSERVATIONS

Beginning 8 June 2008, all observed beetles from each of the six study sites were captured, marked, and photographed. The left, right, dorsal, and ventral sides of all beetles were photographed using a digital SLR camera (Nikon D200 and Canon Rebel XTi) with a macro lens. A 2-mm micrometer was included in each image for scale. Measurements of elytra length, pronotum width, thoracic horn length, clypeal horn length, and distance between the tips of the thoracic and clypeal horns (horn gap) were obtained from the photographs in ImageJ (Abramoff et al. 2004; Fig. 1A). Elytra length and pronotum width are reliable indicators of body size in most coleopterans. Thoracic and clypeal horns, which are formed from the thorax and head body segments, respectively, have been shown to be important in male–male combat (Conner 1988, 1989b, a). The gap between these two sets of horns is used in combat to secure opponents and lift them off of females or fungal brackets (Formica, pers. obs.). Beetles were released at the coordinates of their capture within 36 h. Upon first capture, fluorescent identification tags with a unique three-letter code were affixed to each beetle's elytra using Tuffleye light-cured acrylic (Wet-a-Hook Technologies ®, San Antonio, TX).

In total, 467 beetles (222 males, 245 females) from six demes were captured and phenotyped. All five of the characters that we measured were highly correlated in our metapopulation (0.80–0.95, all correlations were significant; $P < 0.001$). Therefore, we used an unrotated principal component analysis to reduce the dimensionality of our dataset. For selection analysis, we used first principal component (PC1), which explained 89% of the variance. PC1 was loaded equally with each of the five characters and thus can be interpreted as a measure of body size (Table 1) and will be referred to as such hereafter. Investigations of selection on PC2 did not reveal significant selection and are not reported herein.

Behavioral data were recorded during four time blocks, 2100–0100 h, 0300–0700 h, 0800–1200 h, and 1300–1700 h (from 22 June to 20 July 2008). Each study site was visited once per time block and was scanned in both ultraviolet and white light. The location, sex, behavior, and mating partners of each beetle were recorded. Over the course of the study, each deme was visited 10 times per time block, for a total of 40 visits per deme.

Table 1. **Unrotated first principal component (PC1) loadings for phenotypic characters in the metapopulation of *B. cornutus.***

	PC1
Elytra Length	0.45
Pronotum width	0.45
Thoracic horn length	0.46
Clypeal horn length	0.45
Horn gap	0.43
Percent of variance explained	89.0

SOCIAL ENVIRONMENT

A male's social environment was defined as the mean phenotype of the individual's social group, weighted by the number of potential interactions between males ($\overline{\mathrm{PC1}}'$). A geographic approach using kernel density estimator methods was employed to define social groups; the details of this method are described elsewhere (Formica et al. 2010). Briefly, a probabilistic home range was created for each male with at least three behavioral observations that predicted the area where he spent the majority (65%) of his time on the surface of the log. Any male that was observed within this home range was considered a member of the focal male's social group. Conversely, a male was considered part of a focal male's social group if the focal male was observed within the other's predicted home range. This rule ensured that group membership was always reciprocal if both males had definable home ranges; that is, if male 1 was in male 2's social group, male 2 was also in male 1's social group. If a male did not have a definable home range (Formica et al. 2010), he was not considered as a focal male but could still be considered part of another focal male's social group.

To ask whether focal males experienced different phenotypic social environments, we used linear mixed models with the mean body size of social partners as the dependent variable and deme and focal male identity as random effects. For this model, each potential interaction was included as an individual observation. Statistical significance of the random effect was tested using a likelihood ratio test. This and other statistical analyses were conducted in JMP 7 (SAS Institute Inc, Cary, NC).

To determine if the body size of a focal male covaried with the average body size of its social environment, we added focal male body size to the above models as a covariate. To estimate the standardized interactant covariance ($C^{ij'}$) we calculated the Pearson product-moment correlations between the body size of the focal male and the weighted mean body size of its social group ($C^{11'}$; Table 2). To examine whether population subdivision affected $C^{11'}$, we performed this analysis separately for each

Table 2. Population characteristics of six wild demes. Bold values denote $C^{11'}$ whose P values are below 0.05. $C^{11'}$ is the covariance between the body size of focal individuals (PC1) and the mean body size of their social partners ($\overline{PC1}'$).

Density Classification	No. of beetles in pop.	No. of males (no. of males with social groups)	Total no. of brackets	Sex ratio (males/ total)	No. of males/ bracket	Mean body size of focal males	Mean body size of all social groups	$C^{11'}$
Low	31	10 (4)	18	0.32	0.56	0.267	−1.111	**−0.993*** ($F = 13.00$, df = 14.0, $P = 0.003$)
Low	44	20 (15)	23	0.46	0.87	0.562	0.788	**−0.828*** ($F = 34.98$, df = 4.79, $P = 0.002$)
Low	55	28 (24)	40	0.51	0.70	0.184	0.317	**−0.820*** ($F = 86.19$ df = 14.6, $P = 0.003$)
High	93	43 (37)	37	0.46	1.16	0.198	0.053	−0.130 ($F = 0.714$, df = 30.0, $P = 0.405$)
High	131	67 (52)	53	0.51	1.26	−0.356	−0.132	0.014 ($F = 0.019$, df = 47.0, $P = 0.890$)
High	36	20 (13)	16	0.56	1.25	0.517	1.007	−0.221 ($F = 0.60$, df = 9.28, $P = 0.460$)

of our six subpopulations. We also looked for relationships between deme-specific measurements of $C^{11'}$ and two of the likely contributing factors to this value, density (Conner 1989a) and sex ratio, using multiple regression. Density was measured as the number of males per bracket, and sex ratio was measured as the proportion of males in each deme.

SELECTION ANALYSIS

We measured selection using two estimated components of male fitness, copulation success and survival. Copulation success was scored as the number of observations in which a male was observed guarding a female and reliably indicates that a male has copulated successfully (Conner 1988, 1989b, 1995). Copulation success ranged from 0 to 7, with a mean of 0.53 ± 0.08 SE.

To determine overwinter survivorship, all patches of fungus within 100 m of each of the six demes were searched at least three times at the beginning of the next breeding season (May 2009) for tagged individuals. A male was assigned a survivorship of 1 if recaptured and 0 if not recaptured (mean survivorship = 0.45 ± 0.49 SE, cf. Brown and Bartalon 1986). Previous work in our population using maximum likelihood mark–recapture analysis has shown that the recapture probability is very high and long-distance movements are rare (Ludwig 2008), suggesting that our measurements of survivorship are accurate.

To estimate nonsocial and social selection gradients, we used a multiple linear regression of each fitness component on focal and social phenotypes (Lande and Arnold 1983; Brodie et al. 1995; Wolf et al. 1999). Each fitness component was transformed to relative fitness by dividing each value by the metapopulation mean. Because residuals from our regression models were nonnormally distributed, we tested the significance of selection gradients using generalized linear models assuming a Poisson error distribution for copulation success and a binomial error distribution for survival. To meet the assumptions of these distributions, fitness components were not transformed to relative fitness for significance testing.

Our regression models included two traits as independent variables (Fig. 2): body size of each focal male, and the weighted means of body size for each resident's social group. Each of these variables was standardized at the metapopulation level to zero mean and unit variance before analysis, so all selection measures

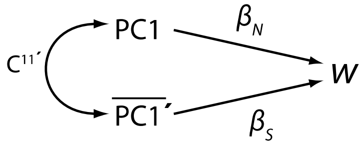

Figure 2. Path diagram illustrating our social selection analysis (after Wolf et al. 1999). Focal individual fitness is regressed on the body size of focal males (PC1) as well as the mean body size of their social partners ($\overline{PC1}'$). Primes denote traits of social partners. Straight arrows represent partial regression coefficients (selection gradients) whereas the curved arrow is the covariance between traits. See text for further definition of terms and interpretation.

are reported as standardized values. The effect of the focal male body size was interpreted as the nonsocial selection gradient (β_N) and effect of the mean social partner body size was interpreted as the social (or group) selection gradient (β_S).

Ideally, we would have performed selection and phenotypic assortment for each deme separately as this is likely the scale at which sexual and social selection occurs. However the population sizes of the demes limited the power of such analyses and necessitated analysis at the metapopulation scale. To investigate deme-level differences, we conducted a posthoc analysis of covariance (ANCOVA) analysis to determine if the density of the individual demes affected the patterns of selection we observed at the metapopulation scale. These analyses were done in ASReml (VSN International) and included deme as a random effect, density (dichotomized as high for demes with > 1 male/bracket and low for demes with < 1 male per bracket), and two interaction terms between density and the focal body size and density and the mean body size of social partners. The results of the ANCOVA analysis and previous findings (Conner 1989a) led us to separate high- and low-density demes and conduct separate selection analyses for each category.

The fitness surface was visualized by fitting a thin-plate spline with the FIELDS package in R (R Development Core Team 2011). We also tested for curvature in fitness surfaces (i.e., nonlinear selection) by adding quadratic terms to our regression models. As we did not detect significant curvature, these results are not reported here.

Results

SOCIAL ENVIRONMENT

Males experienced significantly different social phenotypes during our study; mean body size of social groups ($\overline{\text{PC1}}'$) differed significantly among male focal individuals (likelihood ratio test, $P < 0.0001$;). Focal individual explained 3.1% of the total variance whereas deme explained 4.6%. Across all demes, the body size of focal individuals exhibited a significant negative covariance with the body size of their social group ($C^{11} = -0.158$; $F_{1,119.2} = 8.74$; $P = 0.004$). Stated another way, larger males tended to assort with smaller males than would be predicted by chance and vice versa.

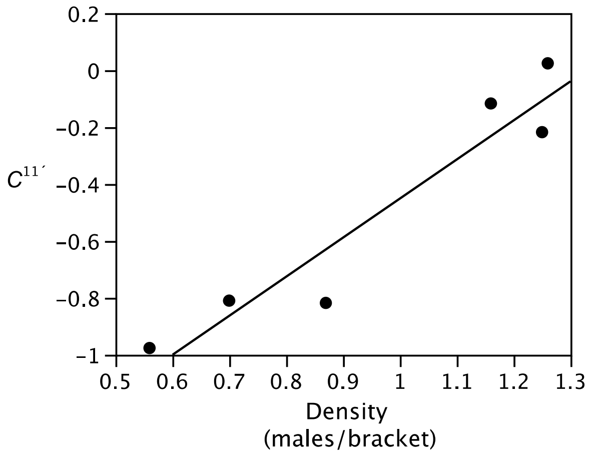

Figure 3. Density predicts the interactant covariance ($C^{11'}$) of individual demes across the metapopulation. The three demes with densities below one male per bracket had interactant covariances significantly different from zero.

When we considered demes separately, we found that $C^{11'}$ varied greatly among demes and was only significantly different from zero in three of six demes (Table 2). Density, but not sex ratio, correlated strongly with $C^{11'}$ across demes (Full model: $r^2 = 0.91$; $P_{\text{model}} = 0.021$; $P_{\text{density}} = 0.018$; $P_{\text{sex ratio}} = 0.563$) with males assorting by body size only in demes with fewer than one male/bracket (Fig. 3). Reanalysis without the smallest deme, which had only four focal males with definable home ranges and a density of 0.56 males/bracket, did not alter this result.

Due to the clear differences in social environments among demes with densities above and below one male/bracket, we conducted a posthoc analysis that pooled individuals within each category (high and low density). The interactant covariance $C^{11'}$ in high-density demes was small and nonsignificant (${C^{11'}}_{\text{high}} = -0.0185$; $F_{1,8.84} = 0.370$; $P = 0.545$), whereas $C^{11'}$ in low-density demes was strong and negative (${C^{11'}}_{\text{low}} = -0.526$; $F_{1,22.87} = 122.55$; $P \leq 0.0001$).

SELECTION ANALYSIS

We detected a strong positive nonsocial sexual selection gradient for body size and a strong negative social selection gradient for the mean body size of social partners (Table 3). In other words, an individual male's expected number of copulations increased with

Table 3. Social and nonsocial selection gradients for copulation success and survivorship. Bold values indicate significant selection gradients.

Type of selection	Trait	$\beta^{(\text{Standardized})}$	χ^2	P
Nonsocial, sexual	Focal body size	**0.378**	9.42	**0.002***
Social, sexual	Mean social partner body size	**−0.356**	7.10	**0.008***
Nonsocial, viability	Focal body size	0.152	2.72	0.099
Social, viability	Mean social partner body size	−0.001	<0.001	0.991

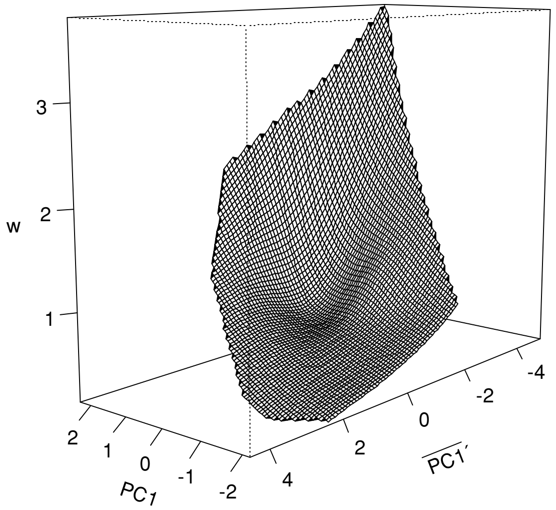

Figure 4. **Social selection surface of focal body size (PC1) and social phenotype ($\overline{PC1'}$) and relative fitness (w); visualized with tension spline. Phenotypic values are standardized to zero mean and unit variance.**

his own body size, but decreased as he associated with larger social partners (Fig. 4). For survivorship, we detected no significant selection gradients, although the nonsocial gradient for body size was nearly significant, suggesting a slight survival advantage for larger males (Table 3).A nearly significant interaction term was detected between density and body size ($F_{1,139.0} = 3.56$; $P = 0.063$), but not between density and the body size of social partners ($F_{1,31.6} = 0.30$; $P = 0.587$).

When pooled high- and low-density demes were analyzed separately, we detected strong significant nonsocial selection on body size only in low-density demes (Table 4). Social selection was nonsignificant in low-density demes, but was strong and negative as expected from the metapopulation analysis. Neither nonsocial nor social selection gradients were detected in high-density demes (Table 4).

At the metapopulation level, the negative assortment of individuals ($C^{11'} < 0$) reversed the negative effects of social selection and resulted in a small net positive contribution to total selection for body size (Table 5). Although social selection gradients were nonsignificant when we split the dataset by density, we also performed this analysis for the low- and high-density demes separately, but draw conclusions with caution. In the high-density demes, social selection had no measurable impact on the magnitude of total selection on body size. However, in the low-density demes social selection accounted for more than 30% of total selection for increased body size (Table 5).

Table 5. **Total selection on body size (PC1) from copulation success (sexual selection).P is the phenotypic variance of the trait expressed in focal individuals, and when multiplied by the natural selection gradient (β_N) quantifies the contribution of natural selection to total selection on a trait. $C^{11'}$ is the covariance between the body size of focal individuals (PC1) and the mean body size of their social partners ($\overline{PC1'}$) and when multiplied by β_S represents the contribution of social selection to total selection (s). See text for expanded explanation of each of these parameters.**

Scale of analysis	$P\ \beta_N$	$C^{11'}\ \beta_S$	s
Metapopulation	0.378	0.056	0.434
High density demes	0.113	0.003	0.115
Low density demes	0.753	0.317	1.092

Discussion

We found that a male forked fungus beetle's copulation success was predicted not only by its own body size, but also by the body size of individuals with which it interacted. This effect of an individual's social group is a form of multilevel selection in which a phenotypic trait has opposing fitness consequences at the individual and group levels. Like other demonstrations of multilevel selection (Stevens et al. 1995; Weinig et al. 2007; Eldakar et al. 2010), our results emphasize the importance of considering how natural selection is impacted by social structure.

Social selection analyses diverge from the usual investigations of multilevel selection by determining how within (nonsocial) and among (social) group selection translate into multivariate phenotypic selection on specific traits. By combining a form of contextual analysis with multivariate patterns of phenotypic assortment, it was possible to evaluate the impact of group fitness effects on the total selection experienced by specific traits in a population. In the case of forked fungus beetles, the form of

Table 4. **Social and nonsocial selection gradients for copulation success (sexual selection) for the entire metapopulation split by high- and low-density demes. Bold values denote significant selection gradients. Both density categories contained three demes each; for high-density category, $n = 102$; for low-density $n = 99$.**

Deme density category	Type of selection	Trait	β (Standardized)	χ^2	P
Low	Nonsocial	Focal body size	**0.775**	9.209	**0.002***
Low	Social	Mean social partner body size	−0.603	2.160	0.142
High	Nonsocial	Focal body size	0.112	0.840	0.359
High	Social	Mean social partner body size	−0.152	1.278	0.258

social selection resulting from the body size of social partners was strong and negative, suggesting that among-group selection should counteract positive individual selection on body size in the metapopulation. However, negative patterns of phenotypic assortment with respect to body size reversed this effect, especially in demes with low densities of males. The net effect of social (or among-group) selection on body size actually enhanced the magnitude of positive selection on body size at the individual level.

SOCIAL AND NONSOCIAL SELECTION

Our analysis revealed that an individual beetle's body size was a strong positive predictor of its copulation success, but not its survival (Table 3). This strong nonsocial sexual selection gradient is consistent with results from previous studies on this species that did not consider social selection. Although Conner (1988) was able to estimate separate selection gradients for elytra length (body size) and thoracic horn length, finding that horn length was under strong positive sexual selection whereas body size was under weaker negative viability selection in this species, high multicollinearity in our dataset precluded effective multiple regression analysis of these traits.

Just as phenotypic variance is required for the action of individual natural selection (Lewontin 1970), variance in social phenotype is the substrate upon which any form of multilevel selection occurs. We found that focal males differed significantly in the average social phenotypes they experienced, even though social associations were somewhat fluid. Furthermore, this variance in social phenotype occurred at the scale of individual focal beetles. This result suggests that individuals may experience multilevel selection effects differentially, rather than solely as common experiences shared by all members of a group.

The average body size of social partners generated a negative social selection gradient via copulation success that was comparable in strength (but opposite in sign) to the nonsocial selection observed for individual body size. This social selection gradient was quite large relative to literature estimates of nonsocial selection (median = 0.16; Kingsolver et al. 2001). The negative effect of partner body size on male copulation success is unsurprising in the context of a mating system such as in *B. cornutus* in which males fight and compete for mates. This pattern may arise because the behavioral phenomena that cause body size to positively affect an individual's own copulation success (e.g., success in male–male combat) concomitantly lead to negative effects on the success of social partners. Larger social partners may be more likely to exclude focal individuals from mating opportunities through direct fights and displacements during mating, or through more indirect effects on temporal or spatial activity patterns that influence the opportunity for mating.

The conflicting directions of social and nonsocial selection detected in our study are similar to results revealed in other systems through contextual analysis (e.g., Tsuji 1995; Banschbach and Herbers 1996; Donohue 2003, 2004; Weinig et al. 2007; Ichinose and Arita 2008). In both *Impatiens capensis* (jewelweed) and *Aquarius remigis* (water strider) selection at the level of the group opposed selection at the level of the individual. In *I. capensis*, larger individuals had higher expected survivorship and greater numbers of cleistogamous petioles (a measure of self pollinated reproduction), yet focal individuals in groups with large individuals had lower expected fitness for both of these components (Stevens et al. 1995). In semi-natural pools, aggressive *A. remigis* males were found in areas with a higher frequency of females, but males in highly aggressive groups were found in areas with fewer females (Eldakar et al. 2010). Such patterns of antagonistic multilevel selection may be common in nature (Tsuji 1995; Aspi et al. 2003; Donohue 2004)—the same trait values that increase individual fitness tend to reduce average fitness of individuals in the group through social interactions among individuals.

THE INTERACTANT COVARIANCE

A major difference between social selection models and other multilevel selection models is the attempt to understand how among-group fitness effects are translated through the multivariate phenotype. In social selection, the interactant covariance ($C^{ij'}$ from eq. 1) links social selection gradients to specific target traits in the focal individual. For example, the same social or among-group selection gradient might have different effects on the net selection experienced by different traits. A trait that does not covary with the social phenotype will not experience any effect of social selection, whereas another trait in the same system that positively covaries with values of the social phenotype would experience proportional effects.

In forked fungus beetles, we detected a significant negative interactant covariance between the body size of the focal individual and the mean body size of the social partners, indicating that large males experienced a mean social group that was smaller than expected by chance and vice versa. Because associating with larger males reduced copulation success, this negative interactant covariance for body size was beneficial for larger males but detrimental for smaller males (Fig. 4). Several nonexclusive behavioral mechanisms could explain this negative assortment of males according to body size. Any process in which size classes of males were attracted or repulsed by other classes could generate such a negative covariance. One example of such a process would be large males avoiding or driving away other large and medium-sized males but ignoring males below a certain threshold. Agent-based modeling simulations have found that relatively simple agents whose patch transition rate was determined by environmental feedback tended to nonrandomly assort by behavioral phenotypes (Pepper and Smuts 2002; Pepper 2007). The findings from these simulations suggest that the behaviors that generate nonrandom phenotypic assortment need not be the result of complex social structure, but rather could result from simple individual-based behavioral rules (see also Aktipis 2004). Assortment may also have resulted from a more indirect mechanism, such as effects of spatial or temporal segregation based on external factors such as habitat quality or distribution of females, although these seem more likely to generate positive rather than negative interactant covariances.

The behavioral mechanisms that generate the critical covariance of interactants are of particular interest for further study. Behaviors that allow individuals to alter their social phenotype either through movement among social groups with different phenotypic compositions or by interacting with social partners in a way that alters the phenotypic composition of their current group (e.g., differential aggression based on the phenotype of the social partner) would allow individuals to influence the selection regime they experience (Formica et al. 2004; Flack et al. 2006; Formica and Tuttle 2009; Oh and Badyaev 2010; Stamps and Groothuis 2010). Such behaviors may themselves represent adaptive responses to social selection.

Although the interactant covariance between body size of individuals ($C^{11'}$) was significant at the metapopulation level, the magnitude and significance of $C^{11'}$ differed substantially among demes. Interactant covariance was strongly negative only in demes with less than one male per bracket. In demes with higher densities, focal males interacted randomly with respect to the phenotypes of social partners. The behaviors that generate this effect are unknown, although it is possible that as density increases, the concomitant increase in the number of interactions among males makes it harder for any form of behavioral association based on phenotype to be maintained. Density-dependent movement among demes could also contribute to this pattern. A previous study on forked fungus beetles detected stronger total sexual selection in lower density demes (Conner 1989a). The weaker sexual selection detected at higher densities in this previous study could be due in part to the absence of interactant covariances in the high-density demes eliminating the effects of social selection.

Conclusions

A complete picture of the total strength of selection (s) on any traits relevant to social interactions must consider the contributions of both nonsocial ($P\beta_N$) and social ($C^{ij'}\beta_S$) selection.

In a conventional multilevel selection framework, our observed negative social selection on body size ($\beta_S < 0$) would be interpreted as selection at the group level acting to reduce the population mean body size. However, a social selection analysis shows that the net effect of group selection on specific traits can be altered by patterns of phenotypic association among individuals. At the metapopulation level, slight negative interactant covariances reverse and reduce the impact of social selection due to the body size of partners. At low densities, this effect is even more dramatic, nearly doubling the net positive selection on body size in the population and reversing the predicted consequence of group selection.

Our results underscore the importance of interactant covariances as a mediator of the effects of social (or group) selection gradients. We predict that patterns of interactant covariance will be influenced by a variety of environmental and demographic factors including density, patchiness, and spatial substructure (e.g., Sinervo and Clobert 2003), as well as a range of behavioral and genetic features such as assortative mating (e.g., Blais et al. 2009), kin grouping (e.g., O'Hara and Blaustein 1985), phenotypic spatial segregation (e.g., Formica and Tuttle 2009), and indirect genetic effects (Wolf et al. 1999; Wilson et al. 2009). Given the prevalence of these phenomena, it is likely that the effects of $C^{ij'}$ and β_S on total selection span the range from strongly antagonistic to strongly synergistic with the effect of nonsocial selection in different natural systems. Quantifying the contribution of social phenotypes to total selection will help us to elucidate the role that these social interactions play in trait evolution and the maintenance of phenotypic diversity.

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