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Sexual selection on locomotor performance in the calanoid copepod *Tigriopus californicus*

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ABSTRACT

Background: A close association between locomotor performance, sexual selection, and fitness has been shown in many species, indicating that reproductive success depends on a complex interaction between locomotor efficiency and mating. Frequently, this interaction produces trade-offs between traits or sexes.

Aims: First, determine whether fitness (fecundity) maximization is traded off with locomotor performance in the context of mating and morphology in *Tigriopus californicus*, a small swimming crustacean. Second, identify if differences occur using different fitness surrogates (egg and offspring production).

Methods: In 110 males and females, we measured egg and offspring production per female, and a suite of attributes: swimming speed, body size (total length), and body elongation (body length/width). We computed trait repeatability of swimming speed and fitness surfaces using cubic splines and selected the best model using stepwise and AIC criteria.

Results: We found contrasting results using egg production versus offspring production as a fitness surrogate. Using egg production, the only target of selection was female size and subtle effects such as stabilizing and correlated selection were detected later, after measuring offspring production. Directional selection suggested that larger and faster males are more efficient in capturing females. Correlational selection between morphological attributes suggested that selection promotes opposite values of elongation and body size in females, and synergic combinations of elongation and body size in males.

Conclusions: Egg fecundity as a fitness surrogate could be misleading, as many effects could appear in a later life stage. Sexual selection on locomotor (swimming) performance appears to be important in copepods, because the criterion of fitness maximization differs in males and females. Correlational selection on morphological attributes also differed between sexes and between attributes (within sexes), suggesting several trade-offs.

Keywords: copepods, correlational selection, fitness, locomotor performance.

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† In memory of Rodrigo Scheihing (1978–2012).

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INTRODUCTION

In non-sessile organisms, displacement capacity is crucial. Running (Pruitt and Husak, 2010), crawling (Denny, 1980), flying (Matyjasiak, 2013), and swimming performance (O'Steen *et al.*, 2010) contribute much to successful foraging, mating, and survival in organisms (Irschick *et al.*, 2008). Given that locomotion depends strongly on structure, the impact of locomotor performance on fitness is usually in close association with morphology. These capacities often co-vary with other traits, especially morphological traits, because of design constraints or because they combine successfully in producing offspring.

Organisms in which there is a trade-off between dispersion capacity and fecundity are useful systems to study how selection impacts performance. In many invertebrates, morpho-functional attributes such as body size and/or locomotor efficiency change between reproductive phases (e.g. sexual and asexual reproduction). This fact generates situations where dispersion and fecundity cannot be maximized simultaneously (Dixon and Kindlmann, 1999; Nespolo *et al.*, 2009; Shine *et al.*, 2011).

Here, we study a small intertidal crustacean (*Tigriopus californicus*) whose locomotor (swimming) capacity is likely fundamental to reach new intertidal ponds and/or mates. If variation in mobility is important, then morphological attributes that determine such mobility should be also important. We hypothesized that the degree of body elongation ('elongation', hereafter) is correlated with swimming speed. *Tigriopus californicus* is a small intertidal harpacticoid copepod characterized by a high tolerance to salinity and temperature, inhabiting the scattered ponds of high intertidal and supralittoral areas (Brown, 1991). These ponds constitute discrete and partially isolated microhabitats where the abiotic conditions (temperature, salinity, and water volume) can vary rapidly in the absence of wave splash or precipitation (Davenport *et al.*, 1997). *Tigriopus californicus* shows great variation in swimming speed and body sizes, and given that eggs are maintained within the body of the female until hatching, larger (and also slower) females potentially produce more eggs. Like other species of copepods, *T. californicus* form tight physical associations between males and females prior to copulation, where males clasp immature females, keeping them attached over several hours until the female is sexually mature, at which time the male fertilizes her (Kiorboe, 2011).

In this study, we tested how morphological attributes (body size and elongation, determined by the length/width ratio) and locomotor capacity (maximum swimming speed) combine to be potential targets of selection. We measured fitness in each pair as a unique reproductive event by counting the number of eggs and live nauplii produced by each pair. Comparatively fast males can capture females more easily than slow males, but bigger (and slower) males could be more efficient in keeping females attached, thus generating a potential trade-off. Females, on the other hand, would experience positive directional selection, as larger individuals can contain more eggs, as happens in many animals (Dahlhoff *et al.*, 2008; Socha, 2008; Sichlau and Kiorboe, 2011; Weber *et al.*, 2011; Nicolai *et al.*, 2012).

MATERIALS AND METHODS

Tigriopus californicus is a harpacticoid copepod living in the intertidal ponds of Chile (Medina *et al.*, 2008) and California (Pritchard *et al.*, 2013). The species is sexually dimorphic; males tend to be slightly smaller than females and have transformed antennules used to grasp females (Pritchard *et al.*, 2013). Males clasp immature females in copepodid stages II–V, using their first modified antennules, and fertilize them when the females reach maturity (stage VI) (Burton, 1985). Fertilized females are then released and keep the eggs attached to the 7th and 8th segments of the abdomen in the egg sac. The females of *T. californicus* only mate once and store the sperm, producing multiple clutches of offspring during their lifetime (Burton, 1985). Clutch size ranges from 20 to 30 per sac (at 18°C), and the babies hatch directly from the egg sac.

Several hundred adult *T. californicus* were collected in Valdivia (39°52′32″S, 73°23′56″W), from intertidal ponds with a specially adapted plankton net of 50 μm mesh, and retained in three glass bottles of 250 mL, with 200 mL marine water each. Bottles were maintained in an incubator and temperature was controlled at 18 ± 2°C. Food was provided *ad libitum*, using a mix of cultured microalgae and smashed green kelp. In total, 110 attached pairs were collected from the bottles and each was isolated and placed in an individual petri dish. When the egg sac was produced, the female was placed in a new petri dish containing fresh seawater.

Fitness measurements

Fitness components were measured as the number of eggs in the egg sac and subsequent number of live nauplii produced by each female. In the first case, petri dishes with females with the formed egg sac were placed under a stereomicroscope to count the number of eggs. In the second case, once the clutch of eggs had hatched (one month later), the number of live nauplii (larvae) was counted under a stereomicroscope by pipetting each nauplius into a new dish.

Maximum swimming speed

For speed measurements, we placed each pair in a graduated sediment plaque that included a small graduated (1 mm) swim channel of 3 × 1 cm. After several tests, we ascertained that both individuals and paired copepods swim in a straight line only within the channel, making it possible to readily record the distance covered by this straight-line swim. Individuals that swam less than 3 cm in a straight line were discarded, as were those that swam in circles. Measurements were performed after each pair was split, and before the appearance of the egg sac in females. We have previously observed that individual copepods swim at maximum speed when placed together with another individual of the opposite sex in the same dish. We placed a single individual in the swim channel for 10 min to acclimate it to its new environment, and then placed its mate in the opposite side of the channel, to elicit maximum speed in the focal individual. We recorded the distance covered by the focal individual swimming in a straight line and calculated ‘maximum speed’ (i.e. maximum swimming speed). We repeated this measurement for each individual every 2 weeks (three times) and estimated the repeatability, i.e. consistent individual differences, by means of the intraclass correlation coefficient, τ (Lessells and Boag, 1987; Falconer and Mackay, 1997), which we calculated as τ = (between-individuals variance component)/(between-individuals variance component + within-individual variance component) from linear models in the R package (see below).

Morphometric measurements

After the pairs split and larvae emerged, adult females and males were fixed in alcohol for morphometric measurements. We measured total body length (body size) and body width under a stereomicroscope, and estimated an index of elongation (length/width). Although elongation was computed using body length, it showed independent properties from body size as it was strongly correlated with swimming speed in females, but weakly correlated with body size in both sexes (see Results).

Statistical analyses

All analyses were performed using the R software version 2.10 (http://www.r-project.org/), and traits were standardized to mean = 0 and standard deviation = 1 prior to analysis. Both fitness measures (i.e. the number of eggs and the number of live nauplii produced by each pair) were standardized to relative fitness by dividing by the mean fitness of the sample. We used the statistical package *lm* [linear models (Crawley, 2007)] to analyse both fitness measures. In each case we started the analysis with a full model that included the following variables: male maximum speed, female maximum speed, male size, female size, male elongation and female elongation, and the quadratic term of each variable. Also in the same initial models we evaluated the following interactions: male maximum swim speed × female maximum swim speed, male elongation × female elongation, and male size × female size. We selected a minimum adequate model with a stepwise procedure using Akaike's Information Criterion.

RESULTS

The bivariate associations between traits are shown in Fig. 1. The maximum swimming speed of both males and females was negatively correlated with body size (Fig. 1A, B) and significantly repeatable ($\tau = 0.23$ for males, $\tau = 0.25$ for females, $P < 0.001$). Elongation was weakly correlated with male and female size (Fig. 1C, D). Maximum swimming speed was not correlated with elongation in males, whereas for females swimming speed and elongation were strongly correlated (Fig. 1E, F). There was a strong and positive association between male and female body size (Fig. 2A) as well as between maximum swimming speed of the two sexes (Fig. 2B). No relationship was observed between male and female hydrodynamics (Fig. 2C).

The model that best explained variation in the number of eggs produced by each pair is shown in Table 1. As expected, female size was the best explanatory variable for egg production and hence appeared under positive directional selection. A more complex pattern was observed when fitness was assessed as the production of live nauplii (Table 2). Again, female size was the trait with the greatest impact on fitness, which was under positive directional selection (Table 2). However, now maximum speed appeared also under positive directional selection, i.e. faster males produced higher numbers of offspring (Table 2). In addition, we detected stabilizing selection acting on male size, i.e. males near to the average of body size in the population had higher fitness (Table 2).

In general, correlational selection was detected for combinations of morphological traits but not for combinations of performance/morphological attributes: (1) favouring the opposite combination of body size and body shape in the two sexes (i.e. negative correlational selection; Table 2), and (2) promoting synergistic combinations of elongation in males and females (more hydrodynamic females combined with more hydrodynamic males tends to maximize fitness; Table 2).

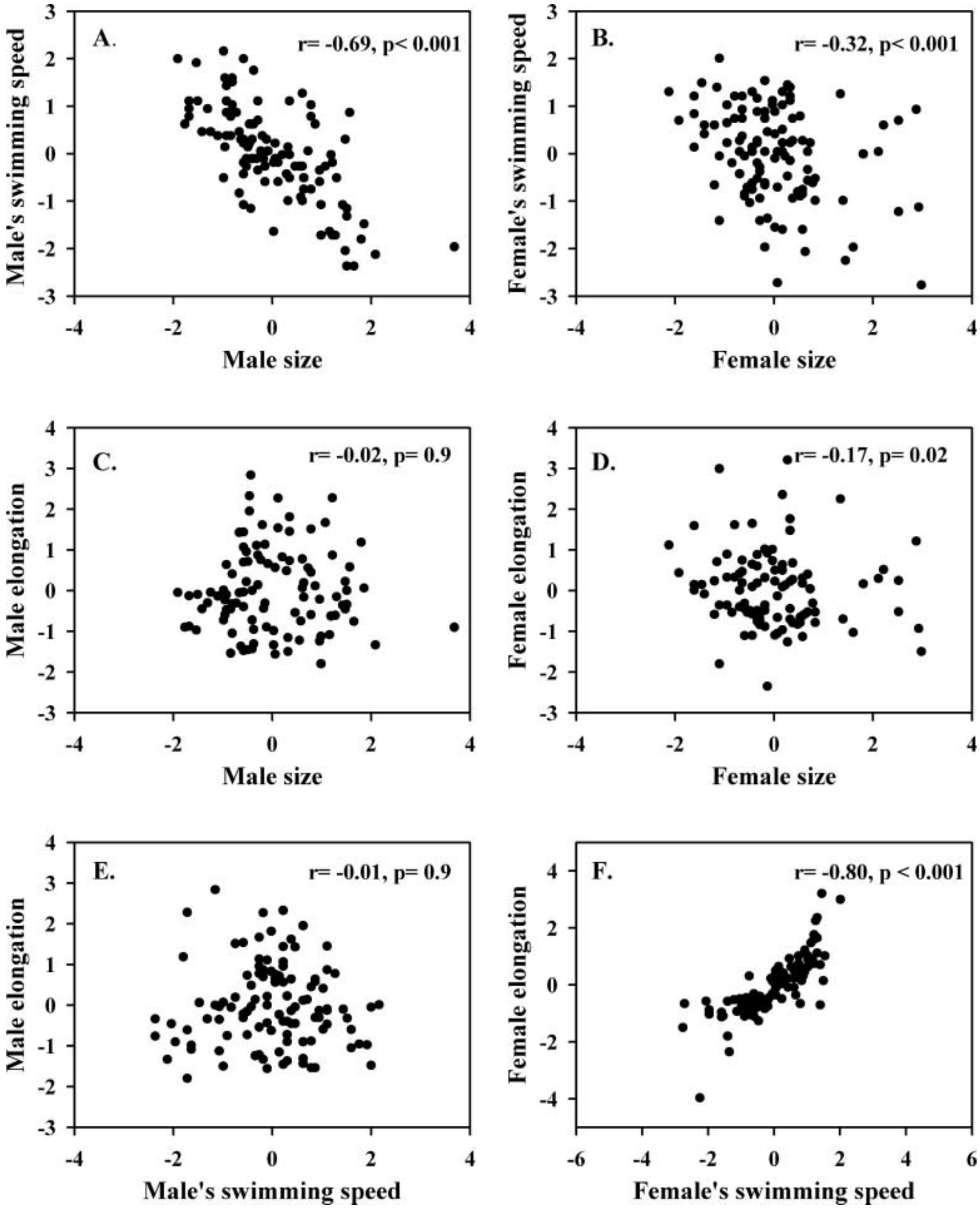

Fig. 1. Phenotypic correlations between body size (total length), elongation (the ratio length/width), and locomotor performance (maximum swimming speed) in males and females of *Tigriopus californicus*.

DISCUSSION

In this study, we assessed the impact of swimming speed (a measure of locomotor performance) on fitness in a swimming crustacean. We used a small intertidal copepod, for which

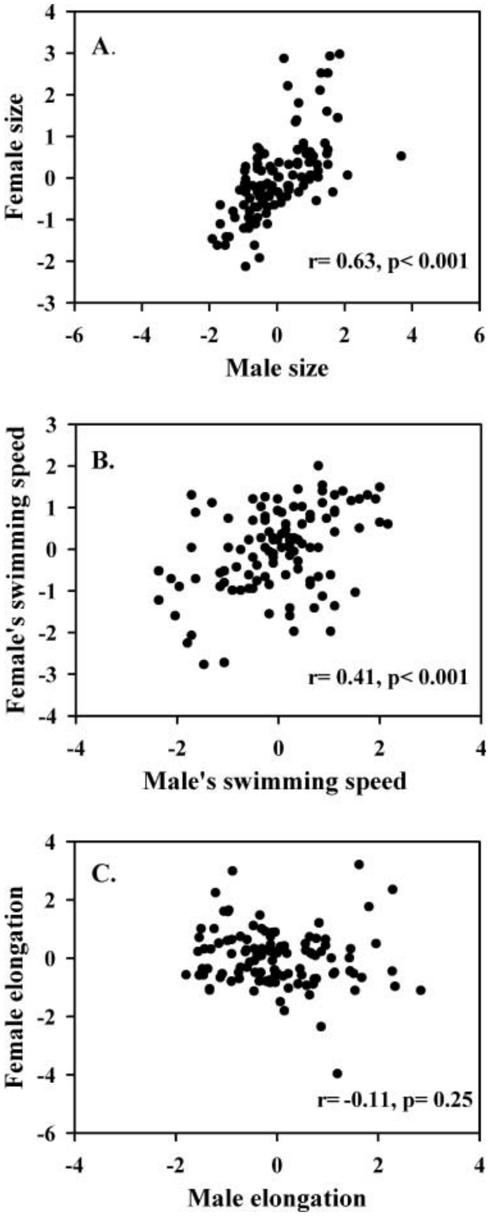

Fig. 2. Bivariate plots showing trait correlations between the sexes in *Tigriopus californicus*. Each data point consists of one pair.

mobility is important for mating success, and likely for colonizing new habitats. We recorded two components of fitness: eggs and offspring (copepodite) production, finding that selection promoted an increase of female size (i.e. positive directional selection) and average body size in males (only using offspring production as a fitness proxy). As initially predicted, maximum swimming speed was repeatable and correlated with morphological

Table 1. Best model from the analysis of selection performed with a linear model (*lm*) using the number of eggs produced by each female as the fitness measure

Coefficient	Estimate ± S.E.	t-value	P-value
Intercept	1.000 ± 0.005	170.347	**< 0.001**
Male speed	0.006 ± 0.006	0.975	0.3319
Male size	0.008 ± 0.008	0.619	0.5373
Female size	0.061 ± 0.006	10.290	**< 0.001**
Male elongation	0.001 ± 0.005	0.876	0.3833
Female elongation	−0.002 ± 0.005	−0.767	0.4451
(Male speed)^2	0.007 ± 0.004	1.814	0.0726
Male speed × male elongation	0.012 ± 0.006	1.780	0.078
Male size × male elongation	0.011 ± 0.008	1.381	0.170
Female size × female elongation	0.007 ± 0.005	1.532	0.1287

Note: All traits were standardized prior to the analysis (mean = 0 and standard deviation = 1). $R^2_{adj} = 0.64$. $\mathrm{AIC}_{\text{full model}} = -329.81$; $\mathrm{AIC}_{\text{reduced model}} = -347.41$. Unless indicated otherwise, each coefficient represents a linear selection gradient. (Male speed)^2 is the quadratic term in the linear model.

Table 2. Best model from the analysis of selection performed with a linear model (*lm*) using the number of live nauplii produced by each female (i.e. divided by the mean of the sample) as the fitness measure

Coefficient	Estimate ± S.E.	t-value	P-value
Intercept	1.015 ± 0.012	86.645	**< 0.001**
Male speed	0.029 ± 0.012	2.520	**0.013**
Female size	0.112 ± 0.011	10.600	**< 0.001**
(Male size)^2	−0.017 ± 0.007	−2.380	**0.019**
Male size × male elongation	−0.028 ± 0.014	−1.984	**0.050**
Female size × female elongation	−0.029 ± 0.011	−2.640	**0.009**
Male elongation × female elongation	0.021 ± 0.009	2.455	**0.016**

Note: All traits were standardized prior to the analysis (mean = 0 and standard deviation = 1). $R^2_{adj} = 0.48$. $\mathrm{AIC}_{\text{full model}} = -151.19$; $\mathrm{AIC}_{\text{reduced model}} = -168.85$. Unless indicated otherwise, each coefficient represents a linear selection gradient. (Male speed)^2 is the quadratic term in the linear model.

attributes such as body size (negatively, in both sexes) and elongation (positively, only in females). Also, body size and swimming speed were correlated between the sexes in a manner suggesting that large, fast males select large and fast females. Moreover, swimming speed was promoted by selection in males, suggesting that faster males are more successful in capturing females. We did not detect correlational selection between maximum speed and morphological attributes. Correlational selection between morphological attributes suggested that selection promotes opposite values of elongation and body size in females and males.

According to Husak and Fox (2008), performance could impact fitness in three ways: (1) by directly influencing mating success; (2) by indirectly affecting survival (which in turn influences mating success); and (3) by simultaneously affecting both survival and mating success. Given that we performed our measurements on isolated pairs, we did not include effects such as predation or inter-individual relationships. In this sense, our results can be only interpreted under the first approach (direct effects of locomotor performance on mating success), which is an important limitation of the study.

Few previous studies have measured locomotor performance in swimming invertebrates. Indeed, of 23 studies and 56 selection gradients of selection on performance reviewed by Irschick *et al.* (2008), only four measured swimming speed. Also, only four of those 23 studies were performed in a non-reptile vertebrate (one in fish, three in amphibians). In addition, our results contribute to two of the central questions in performance studies (Irschick *et al.*, 2008): (1) providing support for the idea that selection is always directional in performance (we found significant directional selection on swimming speed), and (2) not providing support for the idea that selection is stronger for performance than for morphology (we found a steeper directional selection gradient on body size than on swimming speed). Here the specific constraints of swimming performance could be important, which calls for further research.

As it is crucial for many activities that impact fitness, displacement capacity is also highly context-dependent, and costly. As a result, locomotor performance is not always detected under directional selection (Le Galliard *et al.*, 2004, 2013; Irschick *et al.*, 2008; O'Steen *et al.*, 2010). For instance, in reptiles sprint speed could be important for survival but direct selection on this attribute is weak (Calsbeek and Irschick, 2007), because it is modulated by the environment experienced early in life (Le Galliard *et al.*, 2004). In fish, swimming performance becomes important only in the presence of predators (Langerhans, 2009). Here we show that, although we did not measure locomotion costs directly using some energetic measurement, swimming performance was negatively correlated with size, suggesting a potential trade-off at least for males. This trade-off, however, did not appear to offset fitness, as the linear selection coefficient was significant for swimming speed.

Most of the effects were not detected using egg production as the fitness surrogate. This finding suggests that different traits can be under selection depending on the fitness component analysed, and that natural selection can vary in its magnitude and direction depending on the life-history stage addressed (Price and Schluter, 1991; Kelly, 1992). For instance, when we evaluated egg production, only female size was under positive directional selection. However, when offspring production was assessed, the picture became more complex because several other traits, including those of males, appeared under the action of different types of selection. With both egg production and offspring production, we found that female body size was under positive directional selection, with larger females having higher numbers of eggs and live nauplii than smaller females. In addition to the finding of correlational selection (discussed above), faster males produced more offspring compared with other males. Male body size was also under stabilizing selection; in other words, males near to the average of body size in the population had higher fitness than others. Previously, male body size had been related to fecundity probably because larger males produce larger spermatophores containing more spermatozoa (Sichlau and Kiorboe, 2011).

In their review of selection on performance, Irschick *et al.* (2008) encouraged researchers to diversify in terms of the taxa used for performance studies, and especially to explore invertebrate populations. This study provides new data on selection on performance in a small swimming invertebrate, supporting the idea that selection on performance is usually directional, and that it occurs via mating. Further exploration of this system is required, such as experiments to separate the direct and indirect effects of selection (Husak and Fox, 2008) and to determine the criteria of female selection by males, to help explain the correlational selection on elongation we observed in both males and females.

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