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Selection for costly sexual traits results in a vacant mating niche and male dimorphism

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The expected strong directional selection for traits that increase a male's mating ability conflicts with the frequent observation that within species, males may show extreme variation in sexual traits. These male reproductive polymorphisms are usually attributed to direct male–male competition. It is currently unclear, however, how directional selection for sexually selected traits may convert into disruptive selection, and if female preference for elaborate traits may be an alternative mechanism driving the evolution of male polymorphism. Here, we explore this mechanism using the polyandric dwarf spider *Oedothorax gibbosus* as a model. We first show that males characterized by conspicuous cephalic structures serving as a nuptial feeding device ("*gibbosus* males") significantly outperform other males in siring offspring of previously fertilized females. However, significant costs in terms of development time of *gibbosus* males open a mating niche for an alternative male type lacking expensive secondary sexual traits. These "*tuberosus* males" obtain virtually all fertilizations early in the breeding season. Individual-based simulations demonstrate a hitherto unknown general principle, by which males selected for high investment to attract females suffer constrained mating opportunities. This creates a vacant mating niche of unmated females for noninvesting males and, consequently, disruptive selection on male secondary sexual traits.

KEY WORDS: Alternative reproductive tactics, female preference, negative frequency dependent selection, sexual selection.

Competition among males to acquire fertilizations leads to one of the most conspicuous selective forces in evolution, which creates an intriguing diversity of elaborate male ornaments (Andersson 1994). While sexual selection theory predicts strong directional selection for such traits, remarkable discrete variation is often observed within species (Taborsky et al. 2008). Males that invest strongly in costly ornaments may coexist with competitors completely lacking these traits (Gadgil 1972, Gross 1996, Shuster and Wade 2003, Oliveira et al. 2008, Johnston et al. 2013). In principle, when acquiring mates entails high costs, limited resources are allocated using evolved decision rules that maximize reproductive success in the face of inevitable trade-offs. Alternative reproductive tactics (ARTs) evolve when those allocation rules involve mutually exclusive adaptations (Taborsky and Brockmann 2011). If the development of ensuing alternative morphs is based on a genetic polymorphism, negative frequency-dependent selection results in the convergence of fitness of different tactics and, hence, their stable coexistence (Maynard Smith 1982, Sinervo and Lively 1996, Bleay et al. 2007).

In a seminal paper, Gadgil (1972) proposed that investment in costly traits due to intrasexual competition may decrease individual male fitness to an extent such that males not investing in ornaments gain higher fitness. In addition, female behavior (e.g., mate choice) can alter the benefits and costs of male phenotypes and thereby select for alternative male tactics (Alonzo and Warner 2000, Alonzo 2008). However, it is little understood how directional sexual selection for particular traits converts into disruptive selection generating distinct male phenotypes

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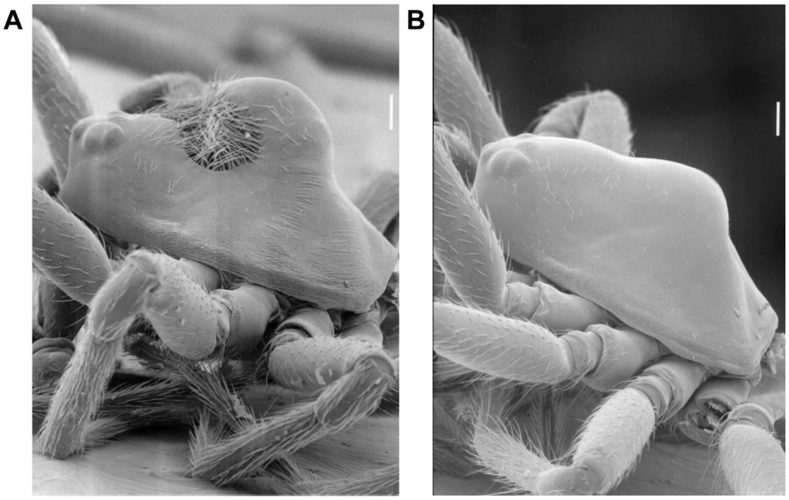

Figure 1. Male head structures *Oedothorax gibbosus*. The *gibbosus* morph (A) has a pronounced hump preceded by a groove with numerous long setae. The *tuberosus* morph (B) lacks this ornament. Scale bar indicates 0.1 mm.

(Maynard Smith 1982, Shuster and Wade 1991, Sinervo and Lively 1996, Shuster 2009). In particular, empirical data demonstrating the emergence of mating opportunities for alternative male tactics because of strong female preference for high investment traits are hitherto lacking.

The polyandric dwarf spider *Oedothorax gibbosus* is a suitable model to investigate the importance of intersexual selection for the emergence and coexistence of alternative male morphs. The carapace of the *gibbosus* morph is characterized by a profound hump and a deep anterior groove with long setae (Fig. 1A). In contrast, the alternative *tuberosus* morph is devoid of these cephalic structures, and its carapace resembles that of females (Fig. 1B) (Vanacker 2004). Previous research revealed that the differences between these male morphs relate to marked divergence in their life history, with *gibbosus* males showing delayed maturity and a shorter lifetime, which strongly suggests that the development of these elaborate traits confers substantial life history costs (Vanacker et al. 2004). Preliminary mating experiments further indicated that males of the *gibbosus* morph are more readily accepted by already fertilized females (Vanacker et al. 2004). This higher acceptance rate of *gibbosus* males by polyandric females is likely due to the extensive production of excretions by glandular tissue in the cephalic hump (Michalik and Uhl 2011). During copulation between a *gibbosus* male and a female, females ingest these excretions, which indicates that it serves as, or mimics, a nuptial gift to persuade females for additional copulations (Vanacker et al. 2003b). The attractiveness of the cephalic excretions produced by *gibbosus* males is further suggested from that observation that also heterospecific males have been shown to feed on this substance (Vanacker et al. 2003c). The inheritance pattern of both morphs is consistent with the expected frequencies of a biallelic locus, with the allele that encodes for the *gibbosus* morph being dominant over that encoding the *tuberosus* morph (Maelfait et al. 1990).

Given the observed female preference for the developmentally costly *gibbosus* traits, this system provides a unique opportunity to (i) test whether the stable coexistence of ARTs can be driven by female preference, and (ii) clarify whether the costs to males involved in producing a trait preferred by females may create a mating niche that can be exploited by alternative males.

Methods

FEMALE PREFERENCE FOR ELABORATE MALE TRAITS

In the first series of experiments, we compared the acceptance rate of both male morphs between fertilized and unfertilized females, taking into account their previous mating experience. Each of 65 *gibbosus* and 68 *tuberosus* unrelated and lab-bred males, whose parents were captured in the "Walenbos" forest in Belgium (50.931°N; 4.879°E), were individually exposed to an unrelated and unfertilized lab-bred female. The number of ensuing copulations was assessed during a 5 h session. Mated females were then divided into two equally sized groups and again exposed to either a naïve *gibbosus* or *tuberosus* male on the next day; the number of copulations was again assessed during a 5 h session. This resulted in four reciprocal mating orders (*gib-gib*; *gib-tub*; *tub-gib*; *tub-tub*), each consisting of 19 pairings. Differences in copulation frequency between male morphs and prior mating experience (morph type) and their interaction were tested by means of a generalized linear model (proc genmod in SAS v. 9.3) using a binomial and Poisson error distribution, and logit and log link function, respectively.

ESTIMATING SPERM PRECEDENCE

Given that females significantly preferred *gibbosus* males after being mated with *tuberosus* males (see Results), we subsequently tested sperm precedence in this *tuberosus–gibbosus* mating sequence. Sperm precedence was estimated by means of the sterile male technique: we sterilized one of the two males used in a mating sequence and determined the proportion of fertilized eggs to estimate the proportion of offspring sired by the fertile male. Males were sterilized by irradiation with 3000rad for 10 min, which was established to give reliable estimates of siring probability (Boorman and Parker 1976, Eady 1991, Harano et al. 2008). Matings were first performed by pairing individual females with irradiation sterilized males (S) only (20 single *tuberosus* matings, four single *gibbosus* matings and eight *tuberosus–gibbosus* mating sequences), which revealed that none of the eggs developed, demonstrating the efficiency of the irradiation treatment. Conversely, matings with 20 control *tuberosus* males and 16 control *gibbosus* males (F) revealed that all eggs developed into juveniles. The proportion of juveniles or eggs could therefore be used as a direct measure of second male sperm precedence in S–F and F–S mating sequences, respectively (Boorman and Parker 1976). All females were allowed to produce two cocoons after mating and second male sperm precedence was estimated from data of both cocoons. Cocoons were inspected after twelve days. At this stage, fertile eggs can easily be distinguished from sterile eggs by the presence of an embryonic spiderling. Individual females were then mated using a *tuberosus* (F)—*gibbosus* (S) ($n = 11$) and *tuberosus* (S)–*gibbosus* (F) ($n = 15$) sequence resulting in a total of 1355 tested eggs over all produced cocoons (average cocoon size: 26 eggs).

Sperm precedence of the second male was estimated by means of a generalized linear mixed model (proc glimmix in SAS v. 9.3) with cocoon order as fixed effect and female, nested within mating order, as a random effect. Significance of sperm precedence was investigated by testing if the proportion of eggs or juveniles deviates from 0.5 in this linear model by means of a one sample *t*-test. To detect if the sterilization treatment resulted in a bias in sperm fertilization ability, we tested whether the proportion of fertile eggs in a S–F sequence differed significantly from the proportion of sterile eggs in a F–S sequence.

Because of the low acceptance rate of *tuberosus* males after females were mated with a *gibbosus* male (see Results), insufficient replicates could be performed of the reciprocal *gibbosus-tuberosus* mating sequence to obtain reliable sperm precedence estimates.

SEASONAL DISTRIBUTION OF MORPHS IN RELATION TO FEMALE REPRODUCTIVE STATUS

Between 77 and 242 individuals were sampled haphazardly by hand at approximately monthly intervals at a field site measuring approximately 200 m², at the Walenbos forest in 2002. The sampling location is a large (>20 ha) and very wet alder (*Alnus glutinosa*) forest where the species occurs chiefly on tussocks of sedges (*Carex* sp.) and in mosses at the base of the alder stems, just above the water level. Individuals were transferred alive to the laboratory and the numbers of juveniles, adult *tuberosus* and *gibbosus* males, and adult females were counted. Adult females were kept individually in plastic vials and fed ad libitum with *Drosophila* until egg cocoons were produced. Unfertilized females can easily be distinguished from fertilized ones by the lack of cocoon production or by the production of pseudo-cocoons, which are cocoons with nondeveloping eggs. We then tested the relationship between the proportion of unmated females and the proportion of *tuberosus* males across the breeding season by means of a Spearman rank correlation analysis.

INDIVIDUAL-BASED MODEL SIMULATION

With help of an individual-based model simulation we investigated if the mating pattern, including the female's change in preference according to her mating status, and the life history trade-offs that we observed in our system effectively result in disruptive selection on male reproductive tactics. Our model describes a population of diploid organisms with (i) the observed trade-off between male investment in sexually selected trait and the life history traits "age at maturity" and "adult life span" and (ii) an increased preference for highly investing males by mated females.

The simulated population consisted of $N = 10.000$ diploid individuals, each with a locus encoding for investment in the evolvable sexually selected trait ($0 \leq I \leq 1$) that is only expressed in males. In line with the observed inheritance of the *O. gibbosus* morph types (Maelfait et al. 1990), we assumed dominance of the allele with largest value, resulting in the investment phenotype $I_P = \max\{I_1, I_2\}$, with I_1 and I_2 being the two alleles at the investment locus. This assumption of dominance is further necessary to allow the evolution of a polymorphism in a random mating population (Rueffler et al. 2006). When both alleles act additively, random mating may impede the evolution of a dimorphism even if disruptive selection favors distinct phenotypic optima (Van Dooren 1999). Allelic variation at this locus has a pleiotropic effect on male phenotype, with larger values of I_P resulting in both the development of more extensive male ornaments allowing males to increase their copulation probability with fertilized females, and in costs expressed in terms of delayed maturity and decreased adult lifespan. In each generation, the population produces N offspring, each inheriting alleles at random from a male and randomly chosen female parent.

The effect of the investment locus on the mating probability of a male was implemented as follows. First, we assumed that the longer adult lifespan of low investment males results

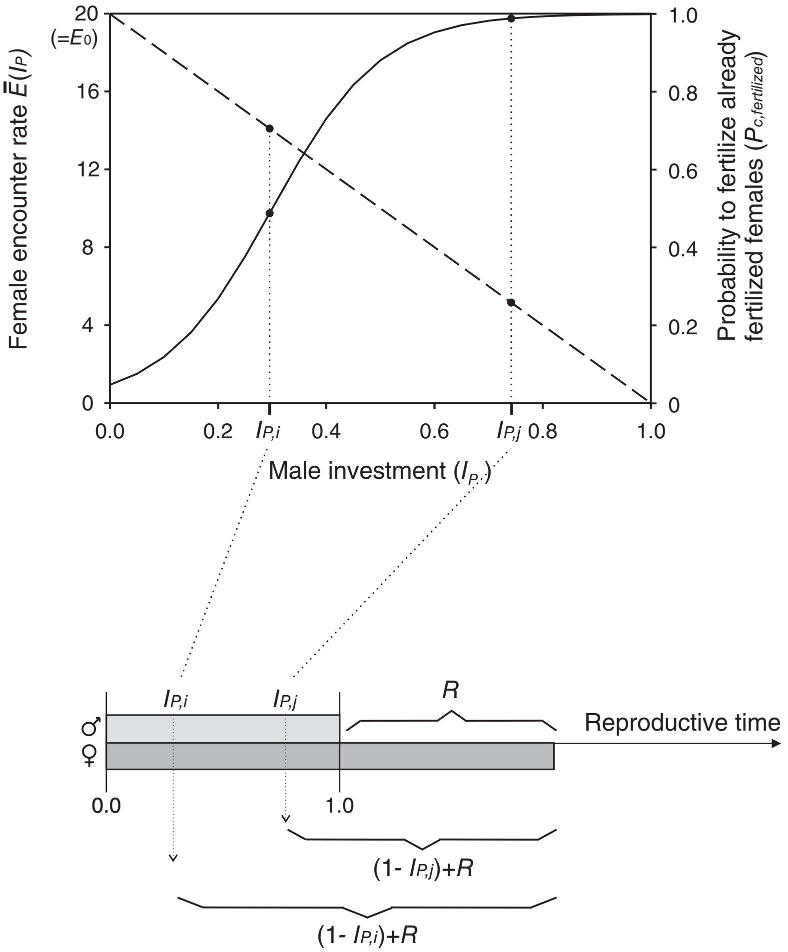

Figure 2. Graphical depiction of the model assumptions and parameters used in the individual-based model simulation. In the upper panel, the dashed line depicts the relationship between male investment, I_P, and the expected number of females a male encounters during its adult lifespan, $\bar{E}(I_P)$, for $E_0 = 20$ (eq. 1). The solid black line depicts the logistic relationship between male investment, I_P, and the probability to fertilize previously fertilized females $P_{\mathrm{c,fertilized}}$ (intercept $a = -3$, slope $b = 10$) (eq. 2). The lower panel graphically depicts parameter R, which expresses the time that females continue to produce eggs after the end of the reproductive period of males, relative to the reproductive period of males. Depicted are the expected female encounter rate, $\bar{E}(I_P)$, the probability to fertilize previously fertilized females, $P_{\mathrm{c,fertilized}}$, and the time of fertilization for two males, i and j, with investment $I_{P,i}$ and $I_{P,j}$, respectively.

in a larger number of females encountered during his adult life (Fig. 2). Hence, let E_0 be the average number of females a male with no investment in sexually selected traits $(I_P = 0)$ encounters, we assumed a negative linear relationship between I_P and the average encounter rate, $\bar{E}(I_P)$, as:

$$\bar{E}(I_P) = E_0(1 - I_P). \quad (1)$$

The effective number of females a particular male encounters, n_{fem}, was then drawn from a Poisson distribution with mean $\bar{E}(I_P)$. Note that according to this relationship, males that invest maximally in sexually selected traits $(I_P = 1)$ will have a female encounter rate, and thus male fitness, of zero.

Males were sequentially assigned to mate with n_{fem} randomly chosen females, with males with low I_P being the first ones to mate to reflect the earlier maturity of low investment males as well as their higher mating rates with unmated females. If the encountered female was unfertilized, we assumed that the male successfully copulates with her. If the female was copulated previously, high investment males had a higher probability of copulation compared to low investment males, according to the following logistic relationship:

$$P_{c,fertilized}(I_P) = \frac{e^{a+bI_P}}{1 + e^{a+bI_P}}, \quad (2)$$

with a and b expressing the intercept and slope of the logistic regression, respectively. Note that following this equation, the quantity $-(a/b)$ corresponds to the I_P value at which males have a probability of 0.5 to copulate with previously inseminated females (Fig. 2).

Under the assumption of complete last male sperm precedence, all offspring of a female will be sired exclusively by the last male if she starts to produce eggs only after males have disappeared from the population. In contrast, if females produce eggs at a constant rate at a time period that (partly) overlaps with the occurrence of fertile males, a male's siring probability will depend on the time interval until the female will remate with another male. More specifically, the siring probability of the last male to mate, *j*, will depend on the survival time of the female after the copulation relative to the time after copulation with the penultimate male, *i* (Fig. 2). This was implemented by defining a parameter *R*, which expresses the time that females continue to produce eggs after the end of the reproductive period of males. Hence, assuming an inverse linear relationship between investment, I_P, and age at maturity, the probability of the last mate, *j*, to sire the offspring of a female under last male sperm precedence, given that she mated previously with male, *i*, was given by

$$P_s(I_{P,j}|I_{P,i}) = \frac{\left(1 - I_{P,j}\right) + R}{\left(1 - I_{P,i}\right) + R}, \tag{3}$$

where $I_{P,j}$ and $I_{P,i}$ denote the investment phenotype, and thus age at maturity, of the last (*j*) and the penultimate (*i*) male to mate with the female. If $R = 0$, the reproductive period of females overlaps completely with the reproductive period of the males. If $R >> 0$, females produce eggs only after the presence of males in the population (Fig. 2).

Lastly, we allowed to vary the strength of last male sperm precedence by specifying *S*, which expresses the probability that the sperm of the last male is used to sire the offspring.

Simulations started with a monomorphic population with $I = 0$, reflecting a population with none of the males investing in sexually selected traits. Alleles mutate with a probability $m_1 = 1/N$, and if a mutation occurs, one of the allelic values of the offspring was added with a mutation effect drawn from a normal distribution with mean = 0 and standard deviation = 0.1. Mutations were only allowed if they resulted in I_P values bound within the interval of [0,1]. We also included a mutation that immediately suppresses the expression of male investment traits. This mutation rate, $m_{2,}$ was also set to $1/N$, and if a mutation occurred, one of both *I* alleles was set equal to 0.

Simulations were initiated with representative parameter values for the encounter rate E_0, *R*, and the coefficients *a* and *b* of the logistic relationship describing the probability to mate with inseminated females as a function of male investment. For E_0, we assumed that males that do not invest in sexually selected traits encounter on average 20 females during their lifetime. This was based on the laboratory observation that when both males and females are placed at field densities (approx. 50 ind/m²), each male interacts on average with 1 female in an 8 h session (F.H., pers. obs.). As *tuberosus* males have an average adult life expectancy of 20 days (Vanacker et al. 2004), our value of $E_0 = 20$ can be assumed to be a reasonable estimate of the number of females a low investment male will encounter during its adult lifetime. Phenological field data and laboratory rearing reveal that females live about twice as long as males, and the extended period of egg laying, *R*, was therefore initially set to $R = 1$. For the logistic relationship parameters, we assumed $a = -3$ and $b = 10$, corresponding to male probabilities to copulate with inseminated females of $P_c = 0.05$ for no investment males, $P_c > 0.99$ for males that invest maximally and $P_c = 0.5$ for males with phenotypic investment trait values of $-(a/b) = 0.3$. To test sensitivity of the outcome toward these parameter values, additional simulations were run with different values of female encounter rates with low investment males ($E_0 = 5, 10, 20, 50$), the timespan during which females produce eggs ($R = 0, 1, 10$ and $1/R = 0$), and the logistic relationship describing a male's copulation probability with fertilized females as a function of his investment in sexual traits ($a = -1, -3, -5$, and -7). We further tested if other genetic systems as the one observed for *O. gibbosus* result in the evolution of a dimorphism by running simulations assuming dominance of the allele with the smallest *I* value, resulting in the investment phenotype $I_P = \min\{I_1, I_2\}$, and additivity of both alleles, resulting in $I_P = \text{mean}\{I_1, I_2\}$.

The evolution of the investment trait was assessed by visual inspection of the dynamics of the distribution of the male investment phenotypes across generations. Simulations were run until no obvious changes in the dynamics were observed, which was generally achieved after 500 generations. The consistency of replicate simulations and effects of the chosen parameter values were checked by averaging the phenotype distribution over the last 1000 out of 2000 generations. For each parameter combination, three replicate simulations were performed.

Results

FEMALE PREFERENCE FOR ELABORATE MALE TRAITS

When exposed to unfertilized females, the mating probability of *tuberosus* males was much higher than that of *gibbosus* males ($X^2 = 10.97$; $P = 0.0009$; Fig. 3). However, when considering only those couples in which mating occurred, *tuberosus* males mated on average once, while *gibbosus* males mated on average twice with the same female in a 5 h session (*n* copulations *tuberosus* = 1.13 ± 0.145 versus *n* copulations *gibbosus* = 2.05 ± 0.242; $X^2 = 11.88$; $P = 0.0006$).

When these fertilized females were subsequently exposed to either one of the two male morphs, *gibbosus* males were accepted with greater likelihood than *tuberosus* males (morph effect 2nd copulation: $X^2 = 9.07$; $P = 0.003$; Fig. 3), irrespective of the male morph with which a female had previously mated (morph

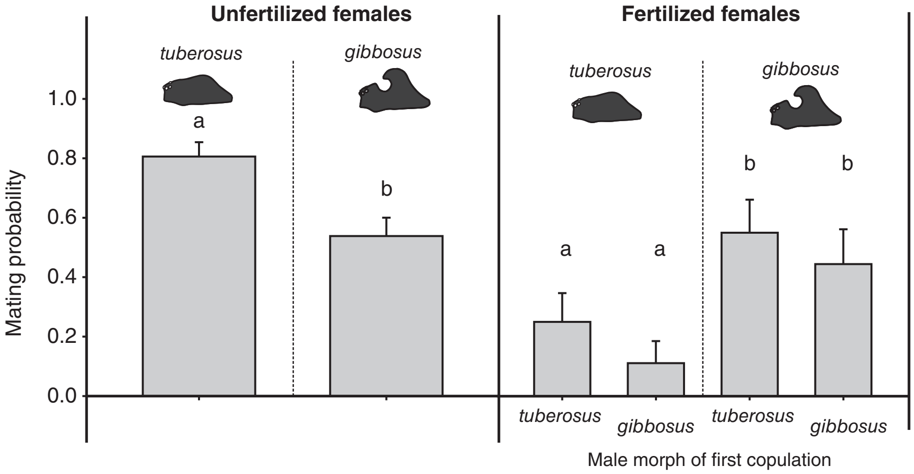

Figure 3. Mean mating probability (±SE) of *tuberosus* and *gibbosus* males when exposed to either unfertilized (left) or fertilized (right) females. Different symbols above bars indicate mean probabilities that are significantly different from each other within each female fertilization status. Sample sizes are $N = 65$ for matings with each male morph for unfertilized females and $N = 19$ for matings with each morph combination for fertilized females.

effect 1st copulation: $X^2 = 1.6$; $P = 0.2$; interaction 1st and 2nd copulation morph effect: $X^2 = 0.25$; $P = 0.6$; Fig. 3).

SPERM PRECEDENCE IN A TUBEROSUS—GIBBOSUS MATING SEQUENCE

When females were first mated with a *tuberosus* male that had been sterilized by irradiation and then with a fertile *gibbosus* male, the proportion of fertile eggs was significantly larger than 0.5 ($P_{\text{fertile}} = 0.67 \pm 0.039$; $df = 23.12$; $t = 4.07$; $P = 0.0005$), indicating significant sperm precedence for the last male in this mating order. Accordingly, when the radiation treatment was reversed, with females being first mated with a fertile *tuberosus* male followed by a sterile *gibbosus* male, a significantly lower proportion of fertile eggs was observed ($F_{1,24.3} = 35.87$; $P < 0.0001$), which was also significantly lower than 0.5 ($P_{\text{fertile}} = 0.28 \pm 0.043$; $df = 25.2$; $t = 4.41$; $P = 0.0002$). Hence, sperm precedence of the second male in a *tuberosus*–*gibbosus* mating sequence was similar for both experiments, equaling a proportion of 0.67 ± 0.039 and 0.72 ± 0.043 for the second male (*gibbosus*), respectively. These proportions were not significantly different from each other (sterilizing order effect: $F_{1,24.3} = 0.62$; $P = 0.44$), indicating that irradiation was unlikely to affect the sperm precedence estimate.

SEASONAL DISTRIBUTION OF MORPHS IN RELATION TO FEMALE REPRODUCTIVE STATUS

The ability of *gibbosus* males to mate with previously inseminated females, combined with their increased paternity rates, should result in strong directional selection for elaborate male traits. However, the development of these traits impose a significant cost to males at the age at which they develop (Vanacker et al. 2004). We therefore tested if the resulting trade-off yields a morph-specific phenological distribution. We hypothesized that the adulthood of *gibbosus* males is delayed to such an extent that *tuberosus* males can exploit the thereby created mating niche by fertilizing freshly emerged (and thus virgin) females.

As predicted by this hypothesis, the relative proportion of both male morphs differed profoundly across the breeding season ($X^2 = 57.61$; $P < 0.0001$; Fig. 4), with the highest proportion of *tuberosus* males occurring when adult females start to emerge, that is before winter (from September onwards) and in early spring (from March until April; Fig. 4A). *Gibbosus* males, in contrast, reach their highest proportion during late spring and summer, that is when the female population consists of individuals that have reached adulthood in early spring (Fig. 4A). To further test if the peak abundance of *tuberosus* males coincides with the appearance of freshly emerged and thus unfertilized females, the proportion of unfertilized females was estimated for each time interval. These unfertilized females can easily be distinguished from fertilized females by their production of unfertile egg cocoons. Plotting the proportion of unfertilized females against the proportion of *tuberosus* males revealed a significant positive association between these proportions across the breeding season ($r_{\text{Spearman}} = 0.68$; $P = 0.015$) (Fig. 4B).

INDIVIDUAL-BASED MODEL SIMULATION

When starting with an initial monomorphic population of males that do not invest in sexually selected traits ($I_P = 0$), the population quickly evolves to a state in which all males invest heavily in costly ornaments that enable them to sire offspring of previously fertilized females (Fig. 5A). Directional selection toward higher investment traits proceeds until the vast majority of males

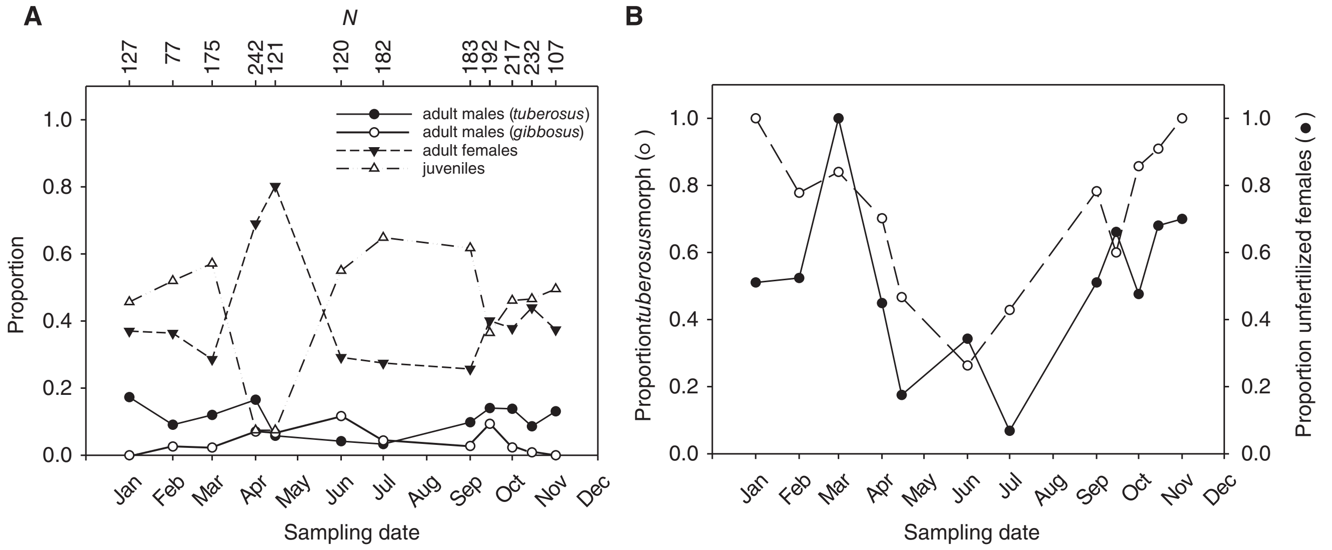

Figure 4. Phenology of *Oedothorax gibbosus* in the field. (A) The proportions of *gibbosus* males (open circles), *tuberosus* males (closed circles), females (closed triangles), and juveniles (open triangles) are depicted for the surveyed population across the year. Numbers on the top axis are the sample sizes during each sampling event. (B) Proportions of *tuberosus* males on the total number of adult males (open circles), and proportion of unmated females on the total number of adult females (closed circles) across the year.

have an investment phenotype of $I_P \approx 0.9$ (generation 90–120 in Fig. 5A). Hence, the ability of males to inseminate fertilized females has a strong selective advantage even if involving a considerable life history cost through the reduction of lifetime, and thus a lower number of females that a male will on average encounter.

Interestingly, when virtually all males in the population invest heavily in their ability to inseminate fertilized females, males that do not invest in such traits quickly invade (from generation 130 onwards in Fig. 5A). This low investment phenotype does not evolve by gradual changes in phenotype space, but by selection of males in which the expression of male elaborate traits was suppressed in a single mutational step. This was confirmed by simulations that allowed low investment males to evolve only by small mutational steps. Here, no invasion of males lacking investment to mate with previously mated females was observed (Fig. 5B). Thus, a population of males investing heavily can only be invaded by males that do not invest and not by males with intermediate I_P values.

Once invasion of no-investment males occurred, the population remained at a stable equilibrium with a clear bimodal distribution in I_P values (Fig. 5A). Replicate simulations showed that at this equilibrium, about 18% of the males do not invest in traits preferred by fertilized females (electronic supplementary material, Fig. S1).

To test the robustness of the observed evolutionary outcome against our chosen parameter values ($E_0 = 20$, $R = 1$ and $a = -3$), simulations were run with different values of female encounter rates with low investment males ($E_0 = 5, 10, 20, 50$), the timespan during which females produce eggs ($R = 0, 1, 10$ and $1/R = 0$), and the logistic relationship describing a male's copulation probability with fertilized females as a function of his investment in sexual traits ($a = -1, -3, -5$, and -7). We further incorporated the strength of last male sperm precedence by specifying the probability that an offspring was sired by the last male ($S = 0.6, 0.7, 0.8$, and 0.9). Under all these scenarios a dimorphism evolved, with variation only in the relative proportion of male morphs, and the mean and variance in the distribution of I_P values of high investment males (electronic supplementary material, Fig. S2).

Assuming genetic recessivity of the allele with the largest I value, which reflects recessivity of the *gibbosus* allele, delayed the onset of evolution of high investment males as mutations resulting in high I values remain hidden under recessivity (electronic supplementary material, Fig. S3, middle panels). Once sufficient alleles with higher I values accumulate in the population, the evolutionary dynamics and equilibrium frequency of high and low investment males is highly similar as observed under the assumption genetic dominance of the allele with the largest I value. As predicted by previous studies (Rueffler et al. 2006), additivity of both alleles hindered the evolution of a dimorphism and only resulted in the evolution of high investment males (electronic supplementary material, Fig. S3, lower panels).

Discussion

The emergence and stable persistence of conspicuous male dimorphisms is a major riddle in evolutionary biology. By integrating data on crucial life history parameters, female preference, and fertilization success of different male morphs in *O. gibbosus* we

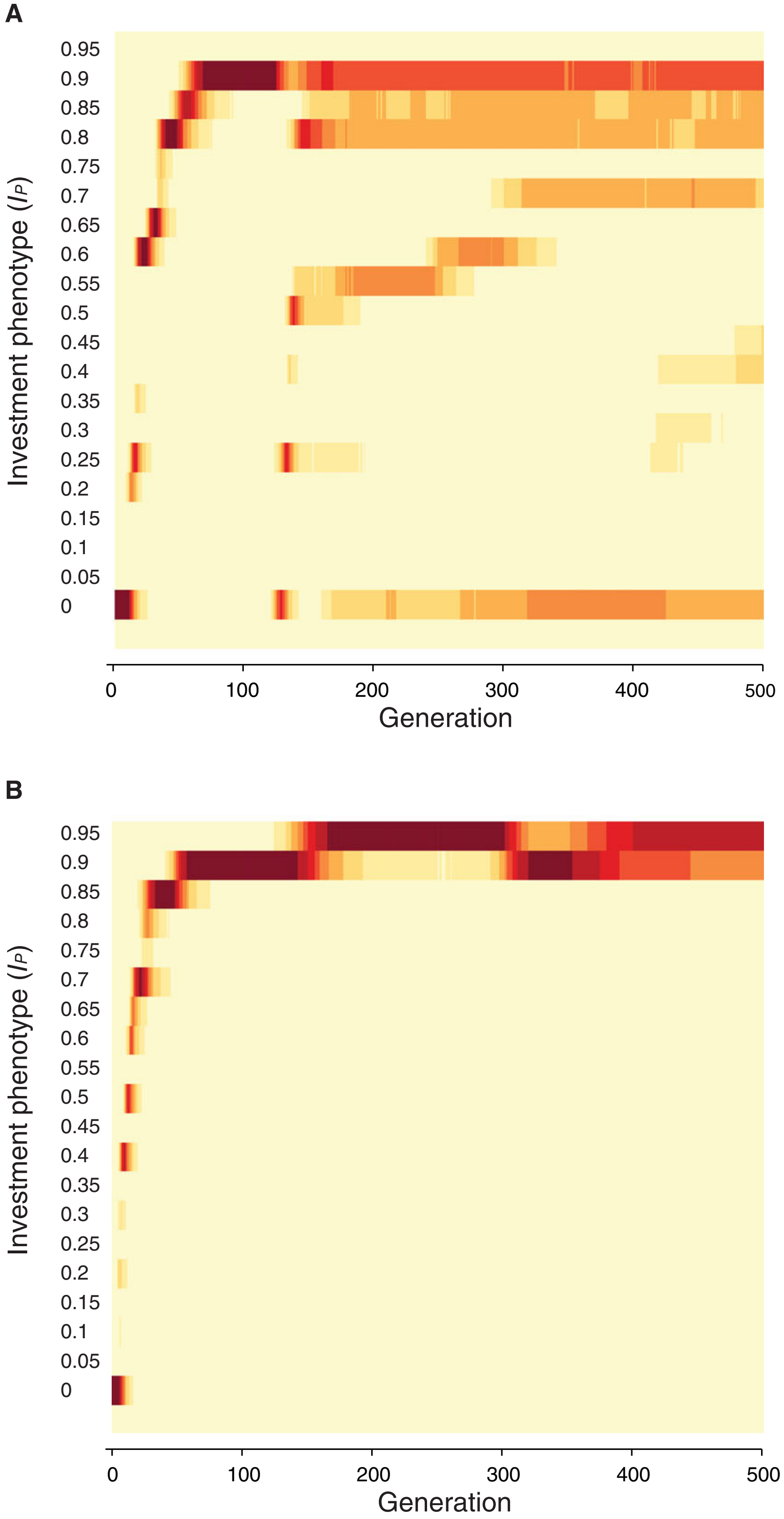

Figure 5. Dynamics of the investment phenotype (I_P), with colors representing high (dark red) to low (light yellow) proportion of individuals of each investment phenotype (I_P) class. Parameter values are: $a = -3$, $b = -10$, $E_0 = 20$, $R = 1$, and $S = 1$ (See *Methods* and Fig. 2 for explanation). (A) Dynamics when the period of females producing eggs partially overlaps with the reproductive period of males ($R = 1$). (B) Dynamics of the investment phenotype distribution (I_P) under the same conditions, but with the mutation rate of alleles suppressing the expression of investment traits, m_2, set equal to 0.

show that intersexual selection for a costly male trait may be so intense that it creates a mating niche consisting of unmated females, that is a vacant mating niche, for males that do not develop such traits. This is corroborated by simulations tailored to our study system, which reveal that mutations suppressing the development of elaborate sexual traits can invade from strong sexual selection for costly traits. Disruptive selection based on female preference can, hence, produce a stable dimorphism in male mating tactics.

First, results presented here together with previous findings of *O. gibbosus* (Vanacker et al. 2004) demonstrate that the *gibbosus* morph exhibiting elaborate male traits is strongly preferred by females, but only after they were previously fertilized by another male, irrespective of their previous mate's morph. The ability of *gibbosus* males to elicit copulations with already inseminated females is probably linked to the elaborate traits characterizing these males' cephalic region. Previous work showed a much higher density of excretory glands in that region in *gibbosus* than in *tuberosus* males (Michalik and Uhl 2011), and female feeding on substances produced in these glands during copulation (Vanacker et al. 2003b). That these substances elicit considerable attraction is further confirmed by the observation that even heterospecific males interact with *gibbosus* males by consuming their secretions (Vanacker et al. 2003c). This was also suggested by our experiments wherein females accepted the same *gibbosus* males on average twice within each mating session, while *tuberosus* males were only accepted once. However, our results also revealed that *tuberosus* males are more likely accepted by virgin females. Although the exact mechanism behind this bias is yet unknown, *tuberosus* males dispose of a particular gland type in the cephalic region that is absent in *gibbosus* males (Michalik and Uhl 2011), which might be involved in female choice.

Directional selection for traits to mate with previously mated females is expected to be particularly favored if it also increases fertilization success with mated females (Pischedda and Rice 2012). Our sperm competition experiments showed that there is last male sperm precedence in a *tuberosus*–*gibbosus* mating order, and that approximately 70% of the offspring are on average sired by the second male to mate. As only 10% of the *tuberosu*s males are accepted by females that have previously mated with *gibbosus* males (Fig. 2), we were not able to obtain reliable sperm precedence estimates of the reciprocal *gibbosus*–*tuberosus* mating order. We can thus not distinguish to what extent the asymmetric fertilization success is due to the morphology of the female genital ducts resulting in primary use of sperm of the last male (e.g., (Foelix 1996, West and Toft 1999)), or to which extent sperm use priority is directly related to the male head structures through cryptic female choice and/or sperm competition (Eberhard 1996, Simmons 2001, Hosken et al. 2008). Recent research on sperm dynamics in spiders indeed uncovered a plethora of mechanisms allowing females to control fertilization (Huber 2005, Herberstein et al. 2011). Information on sperm precedence in this reverse mating order is, however, of less relevance for predicting the evolutionary dynamics in our system. Whether sperm precedence of *gibbosus* males is directly related to the male head structure or due to last male sperm precedence, the increased acceptance rate of high investment males by previously mated females will inevitably result in increased paternity and, consequently, strong directional selection for higher investment. This is also shown by our model, where sperm precedence was only determined by the mating order and not directly by the investment phenotype of the male. As the stochastic nature of our model allows for low probability matings of low investment males after matings with high investment males, which may in these rare cases result in higher sperm precedence of low investment males, high investment males still invaded a population of low investment males, even under more moderate levels of last male sperm precedence ($S = 0.6$). Hence, irrespective of the underlying mechanism, our results show that, if females are exposed to multiple males throughout the breeding season, strong directional selection for complex cephalic modifications that increase paternity rates is to be expected.

Second, previous work demonstrated that the production of these modified cephalic structures imposes considerable life history costs. Under laboratory conditions (18°C), *tuberosus* males mature on average one week earlier (Vanacker 2004) and live up to five times longer than *gibbosus* males under a range of environmental conditions (Vanacker et al. 2003a). This is in line with previous research on the evolution of secondary traits and ARTs, which demonstrated considerable costs of elaborate sexually selected traits (Andersson 1994, Taborsky and Brockmann 2011, Johnston et al. 2013). Assuming that males of both morphs encounter females at similar rates, this reduced longevity of *gibbosus* males imposes a considerable cost in terms of mating success, as they may reduce the number of females encountered by *gibbosus* males to one fifth of the number encountered by *tuberosus* males.

Taken together, these results suggest that investment in traits increasing the probability to sire offspring of previously mated females imposes such high costs on male maturation that mating opportunities may arise for rapidly developing males at the onset of the breeding season. Sampling across the breeding season proved the presence of morph-specific phenological distributions, with *gibbosus* males appearing about one and a half months later in the season than *tuberosus* males. As predicted, *gibbosus* males reach their peak activity when the majority of the female population is already fertilized. Conversely, the highest proportion of *tuberosus* males coincided with the emergence of adult females. These females are still unfertilized, as demonstrated by the fact that they did not produce viable cocoons when bred under laboratory conditions. Given that it takes approximately three weeks before females start to produce eggs after reaching adulthood (F.H., pers.

obs.), *tuberosus* males have ample opportunity to obtain successful fertilizations that will not be overridden by copulations with *gibbosus* males before cocoons are produced. In addition, even if females are attracted by *gibbosus* males during the advancing breeding season, our sperm precedence experiments revealed that still 30% of the offspring are on average sired by *tuberosus* males. In sum, these data clearly show that both morphs exploit distinct mating niches.

Our simulations confirmed that selection on costly male traits can cause vacant mating opportunities that may be exploited by an opportunistic reproductive tactic. Based on the life-history trade-offs observed in *O. gibbosus* and under a wide range of model parameters, our exploration consistently revealed that within a monomorphic population consisting of males that do not invest in elaborate traits, there is strong directional selection for traits allowing males to surpass previous copulations. However, once the majority of males invests heavily in such traits, phenotypic space is created that selects for an alternative tactic that is completely devoid of such costly traits. Both tactics are subsequently maintained by negative frequency-dependent selection. This emergence of a vacant mating niche exploited by males lacking costly traits can be demonstrated most clearly by considering a scenario where females produce eggs only after the activity period of males ($1/R = 0$), in combination with complete last male sperm precedence. This represents a scenario wherein females will only produce eggs fertilized by the last male in a mating sequence. Here, selection for male traits allowing them to access fertilized females proceeds until the average investment phenotype equals 0.94 (Fig. 6), which, according to equation (1), corresponds to an average encounter rate of 1.2 females per male. As this rate includes males encountering females that were fertilized by previous males, a significant part of the female population will not encounter any male. More precisely, the expected number of females that do not encounter any male is defined by a Poisson distribution corresponding to $P(X = 0) = \exp(-1.2) = 0.3$. To scrutinize this situation, we simultaneously monitored the average investment phenotype, I_P, and the proportion of unmated females in the population, when $1/R = 0$ (Fig. 6). These dynamics clearly show that selection for high investment males simultaneously increases the proportion of unfertilized females in the population up to a value of 0.3. This vacant mating niche of unfertilized females can therefore be exploited by a male mutant that does not invest in ornaments, which enables such mutant to mate with many females during his at the same time earlier development and extended adult lifespan. A similar argument holds when the reproductive period of males and females overlap completely ($R = 0$; black bars in electronic supplementary material Fig. S4b). Increased investment will delay the time at which males become mature to such an extent, that sufficient opportunities emerge for low investment males to successfully sire offspring early in the breeding season.

This mechanism also explains the dependence of the evolutionary dynamics on the genetic system underlying the expression of this trait. Our simulations revealed that high investment males evolve by favoring small effect mutations within a monomorphic population of low investment males. Once the population consists entirely of males that invest maximally, a vacant mating niche consisting of unmated females emerges at the onset of the breeding season. Yet, this vacant mating opportunity cannot be exploited by males that invest marginally less than the population average. The slightly earlier development of these males does not allow for sufficient time for the development of their offspring before being surpassed by matings of high investment males. As a consequence, gradual selection for low investment males is unlikely to take place within a monomorphic population of high investment males, and only mutations that result in large mutational effect allow bridging this fitness minimum. This further demonstrates that in the absence of such large effect mutations, the male population is effectively trapped by strong competition without gaining full access to the available mating opportunities (Fig. 5B). Whether this low investment allele is dominant or recessive does not alter the evolutionary outcome, but only affects the rate at which high or low investment males invade. Evidently, additivity of both alleles does not allow for the evolution of a dimorphism. Positively selected large effect mutations that suppress the development of high investment traits will always occur in a heterozygous state, resulting in males with an intermediate investment phenotype, which are negatively selected according to the arguments stated above.

By empirically verifying all necessary components included in the model, we were able to restrict the number of assumptions to a minimum. This approach of using an individual-based model that is tailored to our study system adds a high degree of realism (Kuijper et al. 2012) and therefore provides strong indications that the currently observed female preference pattern, life-history trade-offs and genetic system are effectively expected to result in the emergence of a male dimorphism. It would, however, be interesting to investigate how potential evolutionary feedbacks, such as female coevolution (Alonzo 2008) (either by showing increased preference or resistance against high investment males) or sex-ratio evolution (Fawcett et al. 2011), might affect the future dynamics within such systems.

The evolutionary dynamics observed in our system are in strong congruence with the results obtained by a recent model on the diversifying effect of competitiveness for resources (Baldauf et al. 2014). In their model, individuals compete for resources differing in quality. High competitive individuals attain better resources, but cannot make optimal use of their resources due to the cost of competiveness. For *O. gibbosus*, this could be translated into competitive males (high I_P) being able to access better quality mates, that is females that are less likely to be mated afterwards

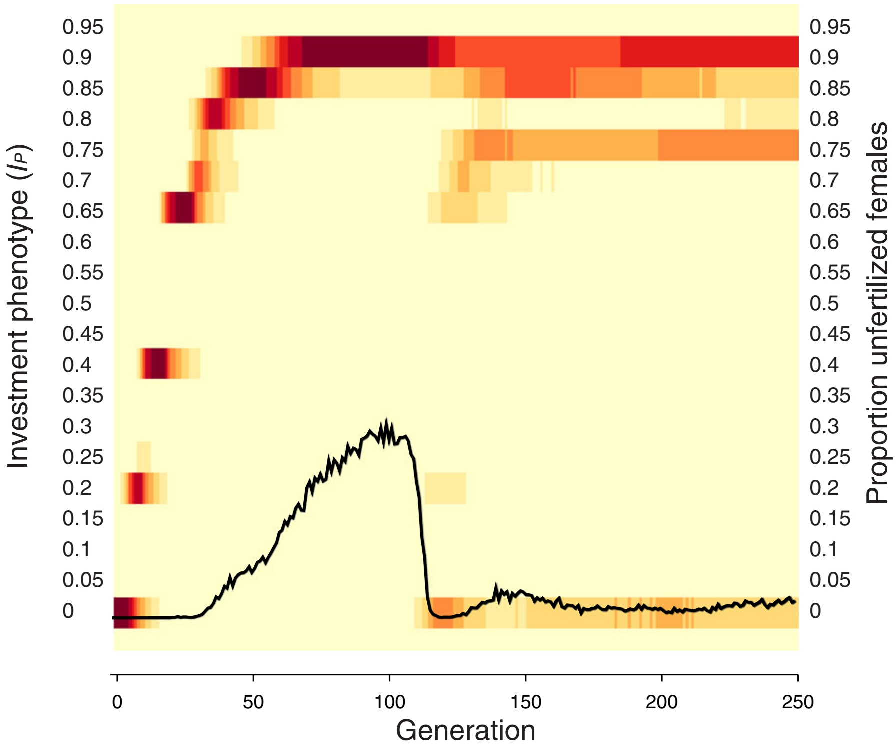

Figure 6. Dynamics of the investment phenotype (I_P), with colors representing high (dark red) to low (light yellow) proportion of individuals of each investment phenotype (I_P) class. Parameter values are: $a = -3$, $b = -10$, $E_0 = 20$, $1/R = 0$, and $S = 1$ (see Methods and Fig. 2 for explanation). The black line depicts the proportion of unfertilized females in the population.

by other males. The cost of investment into this competitive ability is then reflected in their inability to make optimal use of this resource, being reduced longevity and thus a decreased ability to access multiple females. In line with our observations, a dimorphic state evolves first by strong selection for highly competitive individuals. After fixation of highly competitive individuals, weaker competitors quickly invade not by evolutionary branching, but by selection of individuals that invest minimally in their competitive ability to access high quality resources (Baldauf et al. 2014).

In line with previous research on ARTs (Gadgil 1972, Maynard Smith 1982, Gross 1996, Bleay et al. 2007), negative frequency-dependent selection appears the main mechanism driving disruptive selection in male ornaments in this system. This is demonstrated by the observation that invasion of alternative males is most profound when the population consists of a single morph type. Interestingly, invasion of high investment males appeared strong enough to first result in the extinction of low investment males, most likely because during the onset of directional selection for high investment traits no mating opportunities arise for low investment males. Indeed, unfertilized females only appeared when the mean investment phenotype of males is sufficiently high ($I_P > 0.65$, that is from generation 30 onwards in Fig. 6). Although this suggests that *tuberosus* males are the derived state and *gibbosus* males are ancestral, data to confirm this hypothesis are lacking. Current molecular studies based on the mitochondrial markers COI and 16S were unable to resolve the phylogenetic position of the species within the genus (Lopardo and Uhl 2014). Identifying the molecular basis underlying this trait divergence would, however, provide a better basis for reconstructing the evolutionary history of these male cephalic structures.

Conclusions

Previous theoretical research on male polymorphisms focused on the stable coexistence of ARTs and typically assumed only a limited set of tactics (Maynard Smith 1982, Gross 1996, Sinervo and Lively 1996, Oliveira et al. 2008). By explicitly modelling the dynamics of a costly sexually selected trait, our results explain the counter-intuitive observation that a tactic avoiding investment in a sexually selected trait can evolve from strong sexual selection *for* such costly traits. The mechanism proposed here further adds to previous models (e.g., Gadgil 1972, Shuster and Wade 2003) by showing that the invasion of subordinate males relies on the emergence of an unoccupied mating niche created by high investment males that are being “trapped” by strong directional selection. Our simulation results and empirical evidence together provide a first test of the key hypothesis that strong directional selection for elaborate sexual traits may turn into disruptive selection favoring the evolution of an intrasexual dimorphism. This may not only explain extreme male polymorphisms in a wide range of species (Lank et al. 1995, Tsubaki 2003, Wirtz Ocana et al. 2014), but further stresses the importance of mutational and genetic mechanisms in the evolution of intrasexual dimorphisms. Incorporating female preference and estimates of female mating rates with alternative male morphs could therefore provide considerable insight into the importance of vacant mating niches in future studies of the evolution of alternative reproductive tactics.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Stationary distribution of the investment phenotypes.
Figure S2. Effect of changes in E_0 (a), R (b), a (c) and S (d) on the stationary distribution of the investment phenotypes (I_P).
Figure S3. Dynamics of the investment phenotype distribution.