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Y-linked Mendelian inheritance of giant and dwarf male morphs in shell-brooding cichlids

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Behavioural variation among conspecifics is typically contingent on individual state or environmental conditions. Sex-specific genetic polymorphisms are enigmatic because they lack conditionality, and genes causing adaptive trait variation in one sex may reduce Darwinian fitness in the other. One way to avoid such genetic antagonism is to control sex-specific traits by inheritance via sex chromosomes. Here, controlled laboratory crossings suggest that in snail-brooding cichlid fish a single locus, two-allele polymorphism located on a sex-linked chromosome of heterogametic males generates an extreme reproductive dimorphism. Both natural and sexual selection are responsible for exceptionally large body size of bourgeois males, creating a niche for a miniature male phenotype to evolve. This extreme intrasexual dimorphism results from selection on opposite size thresholds caused by a single ecological factor, empty snail shells used as breeding substrate. Paternity analyses reveal that in the field parasitic dwarf males sire the majority of offspring in direct sperm competition with large nest owners exceeding their size more than 40 times. Apparently, use of empty snail shells as breeding substrate and single locus sex-linked inheritance of growth are the major ecological and genetic mechanisms responsible for the extreme intrasexual diversity observed in *Lamprologus callipterus*.

1. Introduction

Mating patterns vary both between and within species [1–3]. If in a species, one sex invests in monopolizing resources essential for reproduction, competitors can exploit such effort by employing alternative reproductive tactics (ARTs) [4–6]. Most often tactic choice responds to current environmental or social conditions, implying phenotypic plasticity [3,7,8]. Yet sometimes ARTs are fixed for life, which may be triggered by an ontogenetic switch [9–11], or can be determined by the inheritance of alleles of large effect [12–15]. In general, however, the genetic architecture of sexually selected trait variation is little known [16,17].

Males in the endemic Lake Tanganyika cichlid fish, *Lamprologus callipterus*, show two distinct life-history tactics and one transient sneaker tactic [18–20]. Large bourgeois males establish nests by collecting empty snail shells that serve as breeding substrate [21]. Previous studies revealed that the exceptionally large body size of bourgeois males in this species results from both natural selection, caused by the manipulation demands of the breeding substrate, and sexual selection on improved competitive abilities [22,23]. When these nest owners spawn, sneaker males of medium size may attempt opportunistic fertilizations by releasing sperm into the shell opening [18,19,21]. Sneaking attempts are rare, and sneaker males are typically younger individuals of the bourgeois male life-history trajectory [18,19]. A second, distinctly different parasitic tactic is performed by dwarf males that wriggle past the spawning female to enter the inner whorl of the shell [18,19]. These dwarf males, which participate in a small proportion of spawnings (see below), release sperm from a privileged position close to the spawning female. However, owing to their small body size dwarf

males have very small testes in comparison with bourgeois males (mean dwarf male gonad mass = 12.4% of mean bourgeois male gonad mass; [19]), which should adversely affect their fertilization success in direct sperm competition with nest owners. Growth data revealed that large bourgeois males and dwarf parasitic males pursue alternative life histories [19,24]. These distinct morphs might be determined by developmental threshold traits involving environmental modifiers, or by inheritance of alleles of large effect.

Sexual selection is usually responsible for strong size dimorphisms among members of one sex [3,25], which may indicate genetic sexual antagonism [26,27]. One possibility to resolve sexual conflict based on antagonistic genetic traits is sex-specific regulation of genes or inheritance through sex chromosomes [28–31]. Here, we test in *L. callipterus* the genetic mechanism responsible for their exceptional intrasexual dimorphism in behaviour, morphology and physiology, and we compare the reproductive performance and success of alternative male types in the field. The data reveal inheritance of male body size and correlated reproductive tactics by a single Mendelian locus on the male sex chromosome, with two alleles determining divergent growth trajectories (cf. [24]). In nature, the two male morphs specialize in very different reproductive tactics, outcompeting each other either in access to mates (bourgeois males) or in sperm competition (dwarf males).

2. Material and methods

(a) Offspring production for pedigree analysis

We used two generations of *L. callipterus* for pedigree analysis to unravel the inheritance pattern of bourgeois and dwarf male phenotypes. Wild-caught males and females ('field sires') or their first offspring generation ('lab-bred sires') produced the F_1 and F_2 generations. All females used to produce the F_2 generation were fish from the F_1 generation (except in one case; figure 1*g*). Offspring were obtained by combining a female with either a bourgeois (10 combinations) or a dwarf male (11 combinations) in a 100-l tank equipped with a filter, a flower pot half and one snail shell as breeding substrate. Spawning occurred 0–75 days after the mates were combined, with no difference in the average time interval until spawning between females combined with bourgeois or dwarf males, respectively. After spawning was completed, the female and the shell with the brood were moved to a 40 l tank, where she cared for the brood until the fry were ready to leave the shell (on average 12 days after egg laying). Then the female was removed and all her fry were kept in the 40 l tank for further four months. Subsequently, 20 haphazardly selected juveniles were transferred into a 100 l tank equipped with a filter and a flower pot half. If less than 20 individuals had survived in one brood, the space for the remaining part of the brood was narrowed accordingly by an opaque partition to standardize density. The water temperature was kept constant at 26°C (±0.5°C) and all fish were fed ad libitum. Offspring were measured once a month (weight, standard and total lengths). The last measures were taken at approximately 2 years of age for the F_1 generation (range 1.15–2.27 years; $N = 10$ broods) and at approximately 1 year of age for the F_2 generation (range 0.97–1.25 years; $N = 11$ broods) and the male type was determined at the last measurement of the experiment (greater than 39 mm bourgeois male type; less than 39 mm dwarf male type [18,24]). The sexes and male types were later confirmed as the fish were kept until the start of reproduction. A total of 21 broods were raised in this way, including seven maternal half-sib broods.

For the pedigree analysis, the frequencies of observed morphs in bourgeois and dwarf male offspring of *L. callipterus* were tested against two autosomal models, with either dwarf or bourgeois sires to be dominant, and a model with linkage to the homogametic sex chromosome (with males being the heterogametic sex). Expected frequencies were calculated from offspring phenotype proportions observed in the field (for bourgeois male offspring 0.9625; for dwarf male offspring 0.0375), assuming Hardy–Weinberg equilibrium (cf. [14]). For each pedigree, the expected and observed frequencies were tested with Fisher's exact test (p-values in table 1), and the overall probabilities were calculated with Fisher's method for combining probabilities (d.f. = 12).

(b) Reproductive parasitism in the field

(i) Samples

One set of samples was collected at Wonzye Point ('WP'; 8°43.5′ S; 31°07.8′ E) near Mpulungu, Zambia. A total of 50 territories of *L. callipterus* were identified and observed in 4–8 m depth (August–December 1996; see [19] for detailed methods). Of these, 10 shells with breeding females were randomly collected for paternity analysis. The other set of samples was collected at Kasakalawe Point ('KP'; 8°46.85′ S; 31°04.88′ E; September–November 2005), which is 8.2 km west of WP. There, the breeding colony of *L. callipterus* consisted of 130 active nests located between 9 and 13 m depth. In this colony, 15 shells with breeding females were randomly collected for paternity analysis. In order to estimate the proportion of parasitized spawnings in the field, of the randomly sampled broods (10 in WP and 15 in KP) fin clips were collected from the nest owner and the breeding female, and the entire fry were collected and stored for DNA analysis. From these random samples, one-third of the fry of each brood were analysed (see the electronic supplementary material, table S1 for the numbers of fry in these broods).

To check for the relative fertilization success of bourgeois nest owners and dwarf males in cases in which the latter successfully entered a shell during spawning, all active nests at KP were checked daily for spawnings using SCUBA diving. As dwarf males are completely hidden inside the shell when participating in spawning, we used the following procedure to determine dwarf male spawning participation: if a spawning was observed ($N = 120$ spawning observations), then the respective shell was marked with a numbered rubber band and visited again 3–6 h later. If spawning had finished (i.e. no more sperm was released by the bourgeois male into the marked shell), the shell containing the eggs and the breeding female was put in a separate net (volume: 2 l) and stored at a depth of 7 m. These nets were checked daily for dwarf males, as dwarf males leave the shell usually some hours after the end of spawning. If a dwarf male was found in the net, a fin clip was taken before he was released where the net was located. A fin clip was also taken of the bourgeois nest owner from which the shell was originally collected. Twelve days post-spawning, the shell was transported to the surface and females and fry were carefully shaken out of it. Before releasing the female, a fin clip was taken for DNA analysis. Fry were preserved as a whole for DNA analysis. Six broods produced with dwarf male participation were collected in this way at KP, and two such broods were collected at WP as described in [19]. This dataset was complemented by two broods with undisturbed dwarf male participation collected in the laboratory, where the fish were kept under semi-natural conditions (figure 3). All fin clips and fry collected in this study were stored in 99% ethanol.

(ii) Paternity analysis

Eleven polymorphic microsatellite loci developed for different cichlid species (loci NP007, NP773, ULI2, Pzeb3, Pzeb4, TmoM5, TmoM13, TmoM25, TmoM27, UME003 and UNH154)

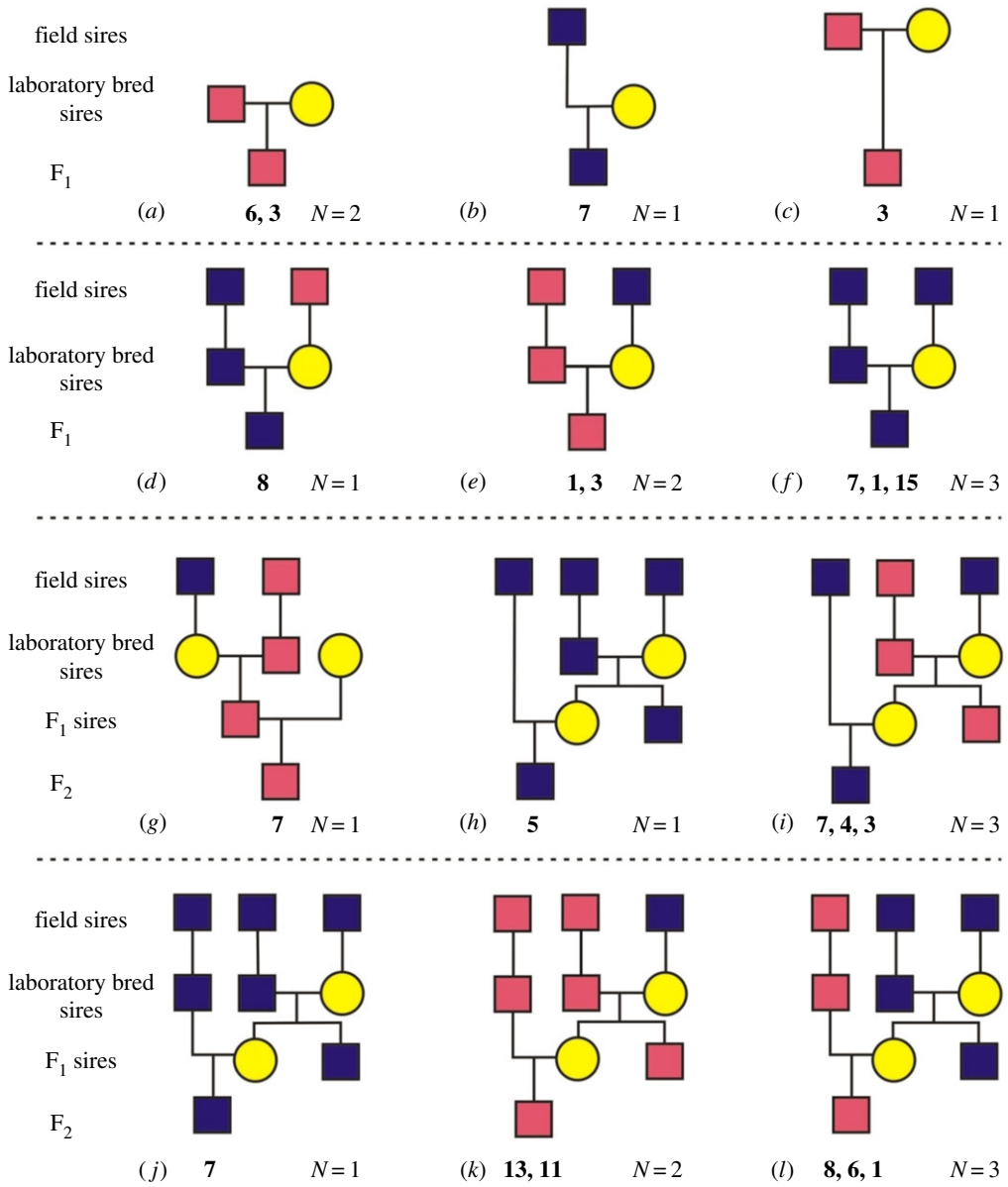

Figure 1. Pedigree analysis of 21 broods with dwarf male or bourgeois male sires. Males represented by squares, females by circles, dwarf males in light red, bourgeois males in dark blue. For each graph, the number of broods with that particular type of pedigree is given in its lower right-hand corner. Bold numbers indicate the number of male offspring obtained in the respective broods. (Online version in colour.)

were used for genetic analysis of broods collected at KP and for the laboratory data. For broods collected at WP, four highly polymorphic loci (NP007, NP773, ULI2 and UME003) were used for analysis. All loci had at least four alleles in 45 unrelated individuals (combined of 18 bourgeois males, 21 females and six dwarf males in the laboratory experiment) and showed independent segregation in all tested groups.

Genomic DNA was extracted from ethanol-preserved fin clip samples of bourgeois males, females and dwarf males (approx. 1–2 mm^2 each) or from whole ethanol-preserved larvae using magnetic beads (MagneSil Blue, Promega). Tissue lysis was done in a lysis buffer containing Nuclei Lysis Solution (Promega), 0.5 M EDTA and Proteinase K according to the Wizard Genomic DNA Purification Kit protocol (Promega). DNA was captured by adding MagneSil Blue to the lysate and washed two to three times with 80% EtOH. Finally, DNA was eluted in 50–100 µl H_2O.

For polymerase chain reaction (PCR) amplification, all 11 microsatellite primer pairs were multiplexed in one PCR reaction using the QIAGEN Multiplex PCR Kit (Qiagen). PCR reactions were carried out in a 10 µl volume containing 1 µl of the genomic DNA, 1× QIAGEN Multiplex PCR Master Mix (consisting of QIAGEN Multiplex PCR buffer with a final concentration of 3 mM $MgCl_2$, dNTP mix and HotStarTaq DNA polymerase), 0.1 µM of locus-specific fluorescent-labelled forward primer (fluorescent dyes were 6-FAM, HEX, NED and PET; Applied Biosystems) and non-labelled reverse primer. In order to improve allele calling efficiency, the sequence GTTTCTT [32] was added to the 5′ end of the reverse primers (except for NP007, Pzeb4 and UNH154). Amplification was achieved in a 96-well GeneAmp PCR System 9700 (Applied Biosystems) by using the following cycling protocol: 15 min at 95°C; 35 cycles consisting of 30 s at 94°C, 3 min at 57°C and 1 min at 72°C, followed by a final 15 min extension at 72°. Fluorescent PCR fragments were visualized by capillary electrophoresis on an ABI PRISM 3100 Genetic Analyzer and analysed using the GeneMapper Analysis Software v. 3.7 (Applied Biosystems). Allele frequencies were estimated with the Cervus 2.0 program [33]. Allele frequencies, observed and expected heterozygosities, and exclusion probabilities were determined using the CERVUS v. 2.0 software package [33,34].

3. Results

(a) Genetic architecture

In the laboratory crossings, all 126 male offspring from 21 broods exhibited their father's phenotype (figure 1). By

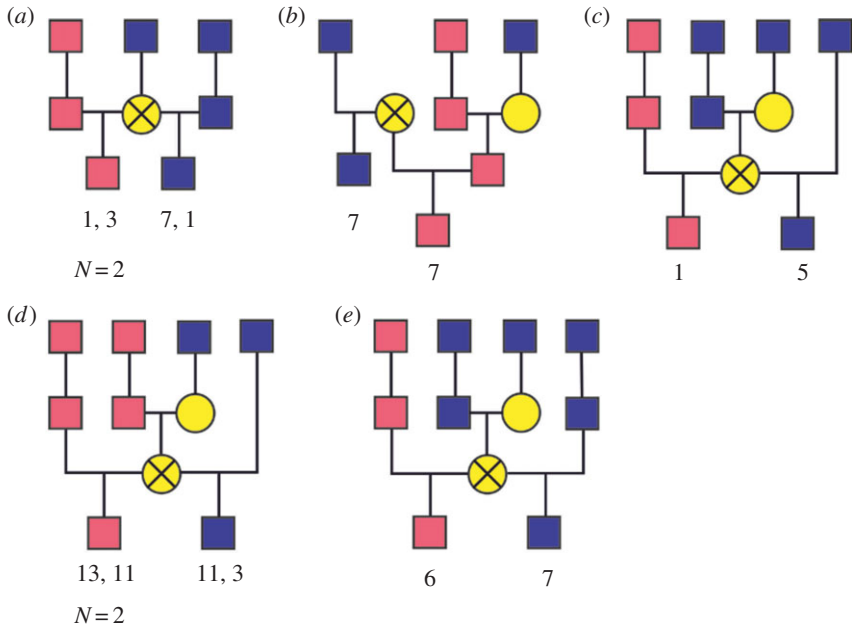

Figure 2. Pedigree analysis of maternal half-sib broods. Males are represented by squares, females by circles. Females mated to a dwarf male (light red squares) and to a bourgeois male (dark blue squares) in subsequent broods are indicated by a cross. Maternal fathers and grandfathers are included in the graphs. For (*a*,*d*), two broods with this pedigree were obtained (females were sisters and mated with different males). Numbers indicate male offspring per brood. (Online version in colour.)

Table 1. Observed and expected frequencies of the dwarf phenotype and 'goodness of fit' tests. Sire type (nest, bourgeois nest male; dw, parasitic dwarf male; unkn., unknown male type); MGF, maternal grandfather; *N* broods, number of broods with that kind of pedigree; *N* male offspring, number of male offspring in these broods; prop. dw offspring, proportion of male offspring belonging to the dwarf phenotype. Expected dwarf male proportions, autosomal inheritance models: DAD, dwarf autosomal dominant model; BAD, bourgeois autosomal dominant model. Sex chromosome inheritance models with male heterogametic sex chromosome XY: XCI = X-chromosomal inheritance model, YCI = Y-chromosomal inheritance model; *p*-values denote differences between the predictions of the respective model and the actual dwarf male proportions determined; *p*-values in the last two rows are 1 everywhere, because the expected dwarf male frequencies in these pedigrees were almost zero; all *p*-values in the last column are 1 because the expected and observed proportions of dwarf male offspring completely matched. *N* = 21 broods with a total of 126 male offspring.

observed					expected proportions of dwarf male phenotype							
sire	MGF	*N* broods	*N* male offspring	prop. dw offspring	DAD	*p*-values	BAD	*p*-values	XCI	*p*-values	YCI	*p*-values
dw	dw	2	24	1	0.635	0.002	0.579	<0.001	0.519	<0.001	1	1
dw	nest	5	19	1	0.510	0.001	0.178	<0.001	0.019	<0.001	1	1
dw	unkn.	4	19	1	0.514	0.001	0.194	<0.001	0.038	<0.001	1	1
nest	dw	4	22	0	0.262	0.021	0.194	0.108	0.519	<0.001	0	1
nest	nest	5	35	0	0.010	1	0.029	1	0.019	1	0	1
nest	unkn.	1	7	0	0.019	1	0.044	1	0.038	1	0	1

contrast, the maternal grandfather had no influence on the male offspring phenotype (figure 1*d*,*e*,*i*,*l*). Pure paternal inheritance was revealed also by maternal half-sib analysis of females mated with males of both phenotypes in consecutive broods. Mothers had no influence on their son's morph ($N = 7$ pairs; figure 2). This suggests that male reproductive types in *L. callipterus* are determined by a single Mendelian locus, with male life-history pathways set exclusively by the father's genotype.

To distinguish whether the locus with alternative alleles determining male morph is located on an autosome or sex chromosome, we calculated expected frequencies of both male types using observed phenotype frequencies from the field for four models of inheritance: two single-locus, two-allele autosomal models with either dwarf (DAD) or bourgeois (BAD) sires to be dominant, and two models with linkage to the sex chromosomes (with males being the heterogametic sex and male type encoded either on the X or Y chromosome; table 1; cf. [14]). These expected frequencies were then tested against the observed frequencies. The overall probabilities for both phenotypes differed significantly from the expected ratios of produced offspring for the three models DAD, BAD and XCI, hence these models must be rejected. The observed inheritance pattern suggests a straightforward single-locus, two-allele polymorphism located on a sex-linked chromosome of heterogametic males (YCI model). Alternatively, the polymorphism could be determined by several loci occurring in a non-recombining portion of the genome. These mechanisms predict that all male offspring correspond to their father's phenotype (dwarf

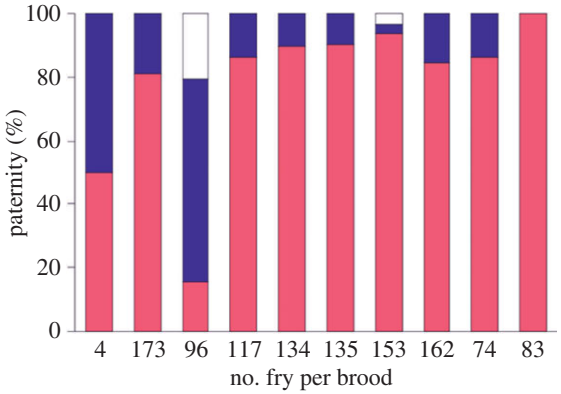

Figure 3. Percentage of offspring sired by different males. Dwarf males (light red), nest owners (dark blue) or sneakers (white). From left to right two broods were collected at WP, six broods at KP and two broods in the laboratory. In all 10 broods all fry were analysed, except for the second brood, where 53 of 173 offspring were sampled. (Online version in colour.)

male or bourgeois male), which was exactly what we found (table 1). The observed paternity of dwarf male and bourgeois male young differs from chance by 1.68×10^{-37} (Fisher's exact probability test).

(b) Reproductive performance of bourgeois and dwarf males

Despite the small body and gonad sizes of dwarf males, when competing with giant bourgeois males for the fertilization of eggs, the majority of offspring were sired by the dwarf male (mean 77.6%; range: 15.6–100%, $N = 10$ broods; figure 3). In two of these cases, other males than the nest owner and the participating dwarf male had sired a few offspring, involving two and three to five sneakers, respectively. These results show that despite constituting only 2.4% of bourgeois male mass on average in nature [19], dwarf males clearly outcompete nest owners in direct fertilization contests.

Compared with this superior success of dwarf males in direct sperm competition with bourgeois nest owners, the relative frequency of this parasitic male morph is low in nature. Dwarf males were found to participate in spawning in only 5% of all surveyed broods (six of 120 broods surveyed for dwarf male participation at KP). To estimate the reproductive success of bourgeois males in comparison to reproductive parasites, we randomly collected 25 broods in the field for paternity analysis (electronic supplementary material, table S1). In 19 of 25 broods, the bourgeois male was the father of the whole brood. In three cases (12% of broods), none of the offspring were descendants of the bourgeois male. In the remaining three cases, a low parasitism rate was detected. On average, 12.6% of fry per brood were not sired by the nest owner (range: 0–100%, s.e. = 6.59, $N = 25$; electronic supplementary material, table S1). When none of the offspring had been sired by the nest owner, this may have resulted from shell stealing from a neighbouring nest after the female had stopped spawning with that neighbour, which happens frequently [35]. Therefore, the three cases in this sample with 100% foreign offspring cannot be safely interpreted as resulting from sperm competition with reproductive parasites. When consequently restricting the analysis to cases where the nest owner sired at least some offspring, on average 0.77% of offspring had been sired by parasitic males in our random field sample (s.e. = 0.45, $N = 22$ broods). This is the combined effect of reproductive parasitism by dwarf males and sneakers, which corresponds well to results obtained by direct behavioural observations of spawning events in the field (1.3%; [19]).

4. Discussion

(a) Disruptive selection caused by breeding substrate

Our data reveal an intrasexual genetic polymorphism responsible for extremely divergent male phenotypes involving morphological, physiological and behavioural differentiation. It is puzzling why in some taxa ARTs are strictly determined by genes, with a complete lack of environmental conditionality, whereas in the majority of cases tactic choice is contingent on information obtained from the environment, either during development or when reproducing [3,6,8]. The ecology of bourgeois and dwarf male reproduction in *L. callipterus* suggests that strong disruptive selection is responsible for the unconditional genetic determination of divergent life-history pathways. This species breeds exclusively in empty gastropod shells [36]. Bourgeois males must pass a threshold size to be able to carry shells [22], which is much greater than the maximum size allowing dwarf males to enter shells with spawning females. In addition, for bourgeois males bigger is always better, as large body size improves shell carrying capacity [22] and competitive superiority [21,23]. By contrast, large dwarf males are unable to enter shells for spawning, hence for them small size is a prerequisite to successfully wriggle into a shell containing a spawning female [19], as confirmed by an interpopulation comparison [37]. As males of intermediate size are less successful and switching between small and large size is impossible, in this situation fixed reproductive tactics are superior, or in other words phenotypic plasticity regarding tactic choice seems maladaptive.

(b) Sex-linked inheritance

The distribution of sex chromosomes is variable in fish. In cichlids, either no sex chromosomes or sex chromosomes with males being the heterogametic sex have been observed [38]. A sex-linked mode of inheritance of male morphs has been suggested also for the poeciliid genera *Xiphophorus* [12,39] and *Poecilia* [40]. In these live-bearing fishes, however, the underlying mechanism apparently involves quantitative rather than qualitative determination of reproductive tactics. In *X. maculatus*, for instance, the number of copies of the melanocortin receptor 4 gene (mc4r), a locus determining the onset of male sexual maturation, is responsible for male size, which in turn influences the prevailing male mating tactic [39]. In accordance with these different genetic mechanisms, in poeciliids male reproductive tactics do not differ distinctly between individuals but rather reflect size-dependent probabilities to either court or force copulations [41]. Male alternative tactics in marine isopod male *Paracerceis sculpta* are apparently caused by the interaction of a major gene with three alleles exhibiting directional dominance, with an autosomal gene and an extrachromosomal factor [42]. In both the ruff *Philomachus pugnax* and the polymorphic damselfly *Mnais costalis*, different male morphs are probably genetically controlled by a single autosomal locus, with two alleles and complete dominance [14,15].

The sex-linked inheritance of male morphs detected in *L. callipterus* is consistent with predictions of population

genetics models anticipating that the genetic correlation between the sexes would be greatly diminished for characters in advanced stages of sexual dimorphism [26]. The proximate mechanism responsible for the enormous size difference between male morphs is their divergent growth speed shown at different life stages [18,24]. Dwarf male sons grow quicker than bourgeois male offspring for the first four months of their lives, but they stop growing altogether at less than 1 year of age. By contrast, bourgeois male sons grow more slowly at the start, but continue to grow indefinitely [24]. Female offspring of both male morphs do not differ in development and growth speed [24], which indicates that the sex-linked inheritance of male morphs established in this study prevents genetic antagonism between the sexes.

(c) Specialized reproductive roles

In external fertilization, a crucial factor determining a male's reproductive success when exposed to simultaneous sperm competition is his relative proximity to females during spawning [20]. Our results suggest that in *L. callipterus*, dwarf males benefit in sperm competition with more than 40 times heavier bourgeois males from their much closer position to the female during spawning within the shell. In contrast to the ejaculates of dwarf males, which are released in direct vicinity of the clutch, the sperm of bourgeois males is released outside the shell entrance and has to pass at least 20 mm before reaching the eggs [19]. This asymmetry in distance that sperm has to overcome before reaching the egg is probably responsible for the biased fertilization success in favour of dwarf males when competing with their vastly bigger competitors.

The superior fertilization success of dwarf males in direct sperm competition with nest owners is offset by the rare occurrence of such parasitic spawning. The roughly 1% of parasitized fertilizations in nature reflects a low parasitism rate compared with other nest-guarding fish species (5–30%) [43]. Highly successful but rare parasitic dwarf males probably reflect the evolutionarily stable equilibrium between these two male life-history pathways [8,44,45].

Genetically determined ARTs are stabilized in a population by negative frequency-dependent selection causing similar lifetime fitness for the different morphs [6,13,44,45]. This is not necessarily the case if ARTs are conditional [6,46,47]. In *L. callipterus*, both conditional (sneaker tactic) and genetically fixed male ARTs (bourgeois and dwarf morphs) coexist within one species, which makes this species an ideal test case for the predictions of evolutionary theory modelling the coexistence of alternative reproductive phenotypes [3,8,44,45].

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References

1. Avise JC, Jones AG, Walker D, DeWoody JA *et al*. 2002 Genetic mating systems and reproductive natural histories of fishes: lessons for ecology and evolution. *Ann. Rev. Genet.* **36**, 19–45. (doi:10.1146/annurev.genet.36.030602.090831)
2. Shuster SM, Wade MJ. 2003 *Mating systems and strategies*. Princeton, NJ: Princeton University Press.
3. Oliveira RF, Brockmann HJ, Taborsky M. 2008 *Alternative reproductive tactics: an integrative approach*, pp. 1–507. Cambridge, UK: Cambridge University Press.
4. Mank JE, Avise JC. 2006 Comparative phylogenetic analysis of male alternative reproductive tactics in ray-finned fishes. *Evolution* **60**, 1311–1316. (doi:10.1111/j.0014-3820.2006.tb01209.x)
5. Taborsky M, Oliveira RF, Brockmann HJ. 2008 The evolution of alternative reproductive tactics: concepts and questions. In *Alternative reproductive tactics. An integrative approach* (eds RF Oliveira, M Taborsky, HJ Brockmann), pp. 1–21. Cambridge, UK: Cambridge University Press.
6. Taborsky M, Brockmann HJ. 2010 Alternative tactics and life history phenotypes. In *Animal behaviour: evolution and mechanisms* (ed. PM Kappeler), pp. 537–586. Berlin, Germany: Springer.
7. Taborsky M. 1994 Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. *Adv. Study Behav.* **23**, 1–100. (doi:10.1016/S0065-3454(08)60351-4)
8. Gross MR. 1996 Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol. Evol.* **11**, 92–98. (doi:10.1016/0169-5347(96)81050-0)
9. Roff DA. 1996 The evolution of threshold traits in animals. *Q. Rev. Biol.* **71**, 3–35. (doi:10.1086/419266)
10. Rowland J, Emlen DJ. 2009 Two thresholds, three male forms result in facultative male trimorphism in beetles. *Science* **323**, 773–776. (doi:10.1126/science.1167345)
11. Tomkins JL, Hazel WN, Penrose MA, Radwan JW, LeBas NR. 2011 Habitat complexity drives experimental evolution of a conditionally expressed secondary sexual trait. *Curr. Biol.* **21**, 569–573. (doi:10.1016/j.cub.2011.02.032)
12. Zimmerer EJ, Kallmann KD. 1989 Genetic basis for alternative reproductive tactics in the pygmy swordtail, *Xiphophorus nigrensis*. *Evolution* **43**, 1298–1307. (doi:10.2307/2409364)
13. Shuster SM, Wade MJ. 1991 Equal mating success among male reproductive strategies in a marine isopod. *Nature* **350**, 608–610. (doi:10.1038/350608a0)
14. Lank DB, Smith CM, Hanotte O, Burke T, Cooke F. 1995 Genetic polymorphism for alternative mating behaviour in lekking male ruff *Philomachus pugnax*. *Nature* **378**, 59–62. (doi:10.1038/378059a0)
15. Tsubaki Y. 2003 The genetic polymorphism linked to mate-securing strategies in the male damselfly *Mnais costalis* Selys (Odonata: Calopterygidae). *Popul. Ecol.* **45**, 263–266. (doi:10.1007/s10144-003-0162-8)
16. Sinervo B. 2001 Runaway social games, genetic cycles driven by alternative male and female strategies, and the origin of morphs. *Genetica* **112**, 417–434. (doi:10.1023/A:1013360426789)
17. Johnston SE, Gratten J, Berenos C, Pilkington JG, Clutton-Brock TH, Pemberton JM, Slate J. 2013 Life history trade-offs at a single locus maintain sexually selected genetic variation. *Nature* **502**, 93–95. (doi:10.1038/nature12489)
18. Taborsky M. 2001 The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. *J. Hered.* **92**, 100–110. (doi:10.1093/jhered/92.2.100)
19. Sato T, Hirose M, Taborsky M, Kimura S. 2004 Size-dependent male alternative reproductive tactics in the shell-brooding cichlid fish *Lamprologus callipterus* in Lake Tanganyika. *Ethology* **110**, 49–62. (doi:10.1046/j.1439-0310.2003.00944.x)
20. Taborsky M. 2008 Alternative reproductive tactics in fish. In *Alternative reproductive tactics* (eds R Oliveira, M Taborsky, HJ Brockmann), pp. 251–299. Cambridge, UK: Cambridge University Press.
21. Sato T. 1994 Active accumulation of spawning substrate: a determinant of extreme polygyny in a shell-brooding cichlid fish. *Anim. Behav.* **48**, 669–678. (doi:10.1006/anbe.1994.1286)

22. Schütz D, Taborsky M. 2005 The influence of sexual selection and ecological constraints on an extreme sexual size dimorphism in a cichlid. *Anim. Behav.* **70**, 539–549. (doi:10.1016/j.anbehav.2004.11.010)
23. Schütz D, Parker GA, Taborsky M, Sato T. 2006 An optimality approach to male and female body sizes in an extremely size-dimorphic cichlid fish. *Evol. Ecol. Res.* **8**, 1393–1408.
24. Wirtz-Ocana S, Schütz D, Pachler G, Taborsky M. 2013 Paternal inheritance of growth in fish pursuing alternative reproductive tactics. *Ecol. Evol.* **3**, 1614–1625. (doi:10.1002/ece3.570)
25. Andersson M. 1994 *Sexual selection*, p. 599. Princeton, NJ: Princeton University Press.
26. Lande R. 1980 Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* **34**, 292–305. (doi:10.2307/2407393)
27. Lank DB, Farrell LL, Burke T, Piersma T, McRae SB. 2013 A dominant allele controls development into female mimic male and diminutive female ruffs. *Biol. Lett.* **9**, 20130653. (doi:10.1098/rsbl.2013.0653)
28. Cox RM, Calsbeek R. 2009 Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *Am. Nat.* **173**, 176–187. (doi:10.1086/595841)
29. van Doorn G. 2009 Intralocus sexual conflict. *Ann. NY Acad. Sci.* **1168**, 52–71. (doi:10.1111/j.1749-6632.2009.04573.x)
30. Mullon C, Pomiankowski A, Reuter M. 2012 The effects of selection and genetic drift on the genomic distribution of sexually antagonistic alleles. *Evolution* **66**, 3743–3753. (doi:10.1111/j.1558-5646.2012.01728.x)
31. Pennell TM, Morrow EH. 2013 Two sexes, one genome: the evolutionary dynamics of intralocus sexual conflict. *Ecol. Evol.* **3**, 1819–1834. (doi:10.1002/ece3.540)
32. Brownstein MJ, Carpten JD, Smith JR. 1996 Modulation of non-templated nucleotide addition by taq DNA polymerase: primer modifications that facilitate genotyping. *Biotechniques* **20**, 1004.
33. Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998 Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**, 639–655. (doi:10.1046/j.1365-294x.1998.00374.x)
34. Kalinowski ST, Taper ML, Marshall TC. 2007 Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **16**, 1099–1106. (doi:10.1111/j.1365-294X.2007.03089.x)
35. Maan ME, Taborsky M. 2008 Sexual conflict over breeding substrate causes female expulsion and offspring loss in a cichlid fish. *Behav. Ecol.* **19**, 302–308. (doi:10.1093/beheco/arm129)
36. Schütz D, Taborsky M. 2000 Giant males or dwarf females: what determines the extreme sexual size dimorphism in *Lamprologus callipterus*? *J. Fish Biol.* **57**, 1254–1265. (doi:10.1006/jfbi.2000.1388)
37. Ota K, Kohda M, Sato T. 2010 Why are reproductively parasitic fish males so small?—influence of tactic-specific selection. *Naturwissenschaften* **97**, 1113–1116. (doi:10.1007/s00114-010-0725-4)
38. Devlin RH, Nagahama Y. 2002 Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* **208**, 191–364. (doi:10.1016/S0044-8486(02)00057-1)
39. Lampert KP, Schmidt C, Fischer P, Volff JN, Hoffmann C, Muck J, Lohse MJ, Ryan MJ, Schartl M. 2010 Determination of onset of sexual maturation and mating behavior by melanocortin receptor 4 polymorphisms. *Curr. Biol.* **20**, 1729–1734. (doi:10.1016/j.cub.2010.08.029)
40. Lindholm AK, Brooks R, Breden F. 2004 Extreme polymorphism in a Y-linked sexually selected trait. *Heredity* **92**, 156–162. (doi:10.1038/sj.hdy.6800386)
41. Farr JA. 1989 Sexual selection and secondary sexual differentiation in poeciliids: determinants of male mating success and the evolution of female choice. In *Ecology and evolution of livebearing fishes* (*Poeciliidae*) (eds GK Meffe, FF Snelson), pp. 91–123. Englewood Cliffs, NJ: Prentice Hall.
42. Shuster SM, Sassaman C. 1997 Genetic interaction between male mating strategy and sex ratio in a marine isopod. *Nature* **388**, 373–377. (doi:10.1038/41089)
43. DeWoody JA, Avise JC. 2001 Genetic perspectives on the natural history of fish mating systems. *J. Hered.* **92**, 167–172. (doi:10.1093/jhered/92.2.167)
44. Gadgil M. 1972 Male dimorphism as a consequence of sexual selection. *Am. Nat.* **106**, 574–580. (doi:10.1086/282797)
45. Bleay C, Comendant T, Sinervo B. 2007 An experimental test of frequency-dependent selection on male mating strategy in the field. *Proc. R. Soc. B* **274**, 2019–2025. (doi:10.1098/rspb.2007.0361)
46. Tomkins JL, Hazel W. 2007 The status of the conditional evolutionarily stable strategy. *Trends Ecol. Evol.* **22**, 522–528. (doi:10.1016/j.tree.2007.09.002)
47. Neff BD, Svensson EI. 2013 Polyandry and alternative mating tactics. *Phil. Trans. R. Soc. B* **368**, 20120045. (doi:10.1098/rstb.2012.0045)