Original Article

Effects of chronic and acute predation risk on sexual ornamentation and mating preferences

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Phenotypic plasticity is widespread in animals. Still, how plastic responses to predator presence affect traits under sexual selection and influence mating preferences is not well understood. Here, we examined how simulated chronic predator presence during development and acute predator presence during mate choice affect the expression of male secondary sexual traits and female mating preference in the three-spined stickleback, *Gasterosteus aculeatus*. Males reared under chronic predator presence developed less intense red breeding coloration but showed higher courtship activity than males that grew up in a predator-free environment. Acute predator presence during mate choice trials did not influence male behavior or ornamentation. Predator presence experienced during development did not affect female mating preferences, whereas acute predator presence altered preferences for male courtship activity. Male body size and eye coloration influenced the intensity of female mating preferences, while the trait changing most in response to predator presence during development (red coloration) had no significant impact. The observed interplay between developmental plasticity in male ornamental traits and environment-dependent female mating preferences may lead to dynamic processes altering the strength and direction of sexual selection depending on both the chronic and acute risk of predation. These processes may contribute to the maintenance of within- and among-population variation in secondary sexual traits, and may, ultimately, facilitate speciation.

Key words: breeding coloration, fish, *Gasterosteus aculeatus*, phenotypic plasticity, sexual selection, three-spined stickleback.

INTRODUCTION

Natural and sexual selection are significant drivers of animal phenotypic diversity (Darwin 1859, 1871), but they often operate in opposite directions (Kotiaho et al. 2001; Dunn et al. 2015). In many species, sexual selection leads to the evolution of conspicuous ornaments or courtship behavior in males, serving to attract females or signal dominance towards rivals (Andersson 1994). Choosy females preferring males based on the extent of their ornamentation are assumed to obtain direct or indirect fitness benefits (Kokko et al. 2003). Because highly ornamented males achieve higher reproductive success, directional sexual selection is expected to favor the spread of genes underlying conspicuous phenotypes, and decreasing genetic variability for these traits within populations (Fisher 1930; Falconer and Mackay 1996). However, the emergence of genes and alleles encoding conspicuous ornaments can be constrained by natural selection via lowered survival probabilities (Kotiaho et al. 2001; Woods et al. 2007; Ercit and Gwynne 2015), and by development and maintenance costs of conspicuous phenotypes (Roberts et al. 2004). Both will lead to a decreased residual

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reproductive value of highly ornamented individuals. Such varying selection pressures resulting from the interplay between natural and sexual selection may contribute to the maintenance of genetic variation (Lewontin 1974; Chaine and Lyon 2008; Cornwallis and Uller 2010; Kuijper et al. 2012; Robinson et al. 2012), and may also drive diversification processes (Maan and Seehausen 2011).

Understanding the causes underlying phenotypic variation in sexual ornamentation and mating behavior within a population is crucial for a comprehensive understanding of the evolution and diversification of secondary sexual traits (Foster et al. 2015b). One mechanism contributing to such variation is phenotypic plasticity, that is, a single genotype's ability to produce different phenotypes in response to environmental variation (West-Eberhard 2003; DeWitt and Scheiner 2004). Phenotypic plasticity has been proposed to facilitate population persistence under varying or unfavorable environmental conditions and contribute to the establishment of populations in new habitats, thereby leading to genetic divergence (Pfennig et al. 2010; Foster et al. 2015a). It may also contribute to variation in sexual ornamentation and mate choice (Griffith and Sheldon 2001; Price 2006; Cornwallis and Uller 2010; Ingleby et al. 2010), and, thus, may affect the outcome of sexual selection and potentially promote speciation processes (West-Eberhard 2003).

Predation risk is an important driver of variation in behavioral, ornamental, and morphological phenotypes (e.g., Endler 1995; Ferrari et al. 2010; Hettyey et al. 2015; Groenewoud et al. 2016). It is a potent selective force acting against conspicuous phenotypes and favoring the maintenance of cryptic appearance (Ryan et al. 1982; Ekanayake et al. 2015), as well as the emergence of anti-predator phenotypes (Brock et al. 2015; Price et al. 2015; Freudiger et al. 2021). Accordingly, in predation-exposed natural populations, reduced ornamentation (Endler 1980; Lindholm et al. 2014; Outomuro and Johansson 2015), and cryptic mating behavior (Koga et al. 1998; Engqvist et al. 2015) occur regularly. Furthermore, life-history theory predicts the evolution of different investment strategies in reproductive traits to be dependent on predation risk (Candolin 1998; Lima and Bednekoff 1999; Wolf et al. 2007). Adaptations to risk might either be genetically fixed or phenotypically plastic. Although predator-induced developmental plasticity is known to alter several characteristics of living organisms, including behavior, morphology, development rate, or toxin production (e.g., Tollrian and Harvell 1999; Relyea 2001; Hettyey et al. 2019), relatively few studies examined its impact on sexually selected traits. In guppies (*Poecilia reticulata*) and a riverine cichlid (*Pelvicachromis taeniatus*), for example, the exposure to chemical predator cues delayed the development of conspicuous male coloration (Ruell et al. 2013; Meuthen et al. 2018). Furthermore, in guppies and palmate newts (*Lissotriton helveticus*), males remained less colorful when chronically exposed to such cues (Ruell et al. 2013; Winandy and Denoel 2015).

In the presence of predators, females have repeatedly been shown to be less selective and show reduced or altered preferences (e.g., Forsgren 1992; Godin and Briggs 1996; Pilakouta et al. 2017). Under risky conditions choosing less conspicuous male phenotypes may be beneficial, as flamboyant sexual patterns might attract predators (Magnhagen 1991; Zuk and Kolluru 1998). This can lead to changes or inversion of mating preferences, for example, from a preference for conspicuous traits to their avoidance (Godin and Dugatkin 1996; Bierbach et al. 2011; Pilakouta and Alonzo 2014). Thus, predation risk can alter the strength and sometimes even the direction of sexual selection. However, little is known about how chronic predator exposure during ontogeny affects the development of ornamental and behavioral traits of males, how it affects female choice, and how the interplay between altered male phenotypes and female preferences ultimately shapes mating patterns (Ruell et al. 2013; Winandy and Denoel 2015).

In the present study, we examined how variation in chronic predator presence during ontogeny and in acute predator presence during mate choice affects traits under sexual selection in three-spined stickleback (*Gasterosteus aculeatus*). As a colonizing species (e.g., von Hippel 2008; Hudson et al. 2021), three-spined stickleback are often the only fish species in a water-body (Dingemanse et al. 2007; von Hippel 2008; Wund et al. 2015). At the same time, the source population might face constant predation pressure from piscivorous fish, and three-spined stickleback are indeed an important food source for a wide range of species (e.g., Maitland 1965; Moodie 1972; Reimchen 1980; von Hippel 2008; Johnson and Candolin 2017). Their name-giving spines and body armor represent morphological adaptations against predation (Hoogland et al. 1956; Moodie 1972; Gross 1978; Reimchen 1994), and variation in this armor has been linked to the level of risk a population faces (Reimchen 1994; Reimchen and Nosil 2004). During the reproductive phase, males develop a conspicuous nuptial body coloration, including a strikingly red belly and bright blue eyes, which plays a role in intra- as well as in intersexual selection (e.g., Rowland 1984; Milinski and Bakker 1990; Bakker and Milinski 1993; Bakker and Mundwiler 1994; Flamarique et al. 2013). This conspicuous coloration, however, makes them more vulnerable to predation (Maitland 1965; Moodie 1972; Whoriskey and Fitzgerald 1985; Johnson and Candolin 2017). Like many other fishes possessing non-structural coloration, three-spined stickleback can change this coloration within a short timeframe (Candolin 1999; Kim and Velando 2014; Hiermes et al. 2016). Populations facing high levels of predation differ from predator-free populations in coloration (Moodie 1972; Gygax et al. 2018), morphology, (Reimchen 1980; Bell and Foster 1994; Marchinko 2009), and anti-predator behavior (Huntingford et al. 1994; Dingemanse et al. 2007; Foster et al. 2015a; Wund et al. 2015). Apart from some contribution of random genetic drift, such among-population differences mainly result from local adaptation (Moodie 1972; Peichel et al. 2001; Reimchen and Nosil 2004) or phenotypic plasticity (Candolin 1997, 1998; Frommen et al. 2011; Kozak and Boughman 2012; Stein and Bell 2014; Ab Ghani et al. 2016; Gygax et al. 2018). This makes three-spined stickleback a great model system to investigate the effect of plastic responses to predator presence on both the development of sexually selected traits as well as preferences for these traits.

We performed a comprehensive test of predator-induced phenotypic changes in male sexually selected traits and female mating preferences in the three-spined stickleback. We examined the effects of (i) chronic predator presence experienced during individual development, and (ii) acute predator presence during reproductive interactions on (a) male ornament expression and (b) courtship behavior, as well as on (c) female mating preferences.

MATERIAL AND METHODS
Experimental subjects

The laboratory-reared population used in this study represents the F1 offspring of fish caught from an anadromous, genetically heterogeneous population from the Island of Texel, the Netherlands (Heckel et al. 2002). The parental generation was collected during

their spring migration in April 2009 and brought to the laboratory at the Konrad Lorenz Institute of Ethology in Vienna, Austria. Here, they were housed under standardized summer light conditions (16 h light, 8 h dark) at a temperature of 17 ± 1 °C to simulate the start of the breeding season. Fish were fed daily with frozen *Chironomus* larvae in excess. The breeding procedures followed the protocol of Frommen et al. (2008). Briefly, males that showed signs of nuptial coloration were isolated in 10 L tanks filled with aerated, aged tap water and provided with sand on the bottom and green cotton wool threads as nest-building material. Once a male had finished nest building, a gravid female was introduced into his tank to allow mating. In total, we used the offspring of eight unrelated stickleback pairs, each pair contributing one clutch. Eggs were removed from the nest within 24 h after spawning and divided into two equal-sized sibling groups. Depending on initial clutch size group sizes varied between families and contained between 20 and 30 eggs. Excess eggs were used for other experiments. Eggs were transferred to small tanks (16 × 10 × 6 cm, l × w × h) aerated by air stones. From the day of separation onward eggs and hatching fry were kept in the respective treatment water (see below). At the age of 3–4 months, we transferred juvenile fish into larger tanks (50 × 30 × 30 cm) filled with the respective treatment water (see below). Each tank was equipped with an internal filter to clean and aerate the water and with halved clay pots for shelter. Tanks were visually separated from each other by green Styrofoam. They were placed on sand-colored paper to reduce stress caused by an overly bright bottom. Tanks were illuminated by fluorescent lamps (36 W) placed above the tanks. The light regime was switched to winter light conditions (8 h light, 16 h dark) during the winter months. In late spring, summer light conditions were re-established to simulate the beginning of the breeding season (Borg et al. 2004). One-third of the water was replaced by treatment water daily. Water quality and fish health were regularly checked, and survival of larvae, subadults, and adults was high and comparable to similar breeding lines (cf., Frommen et al. 2008). We counted all adult fish approx. 3 months after the experiments took place to measure potential survival differences between the treatments. The number of surviving fish did not differ significantly between the chronic control and predator treatment (Wilcoxon matched-pairs signed-rank test, $N = 8$ families, $W = 22.0$, $P = 0.2$).

Treatment water

To simulate predator presence, we used European perch (*Perca fluviatilis*) that occur in sympatry with *G. aculeatus* in large parts of Europe, including the Texel population (pers. communication Jan Hottentot, local commercial fisherman) and that readily prey on juvenile and adult stickleback (Hoogland et al. 1956; Gross 1978). We added perch-conditioned water (see below) daily to the tank of one sibling group. The other group received aged water as a control. Previous studies showed that larval three-spined stickleback readily recognizes predatory perch solely based on chemical cues (Lehtiniemi 2005; Frommen et al. 2011).

When producing the perch-conditioned water, we used a protocol comparable to Frommen et al. (2011). Perch initially measured between 75 and 83 mm standard length (SL) and were kept in tanks (50 × 30 × 30 cm) each housing one individual. These tanks were equipped the same way as the tanks containing the stickleback. We produced aged water without predator cues in identical tanks that differed from the predator treatment only by the lack of a perch. The tanks providing treatment water were aerated through internal filters and were placed on shelves above the stickleback

tanks. We connected each treatment tank to the tanks holding the experimental fish through a hose-and-tap system, which facilitated water exchange. Perch were fed daily with frozen *Chironomus* larvae in excess. Feeding took place after treatment water was flushed to the stickleback tanks to reduce any potential influence of chemical *Chironomus* cues on the outcome of our experiment. Each perch tank and control tank provided treatment water for two unrelated sibling groups of stickleback. Experimental fish were kept under these conditions for approximately one year, when they reached sexual maturity.
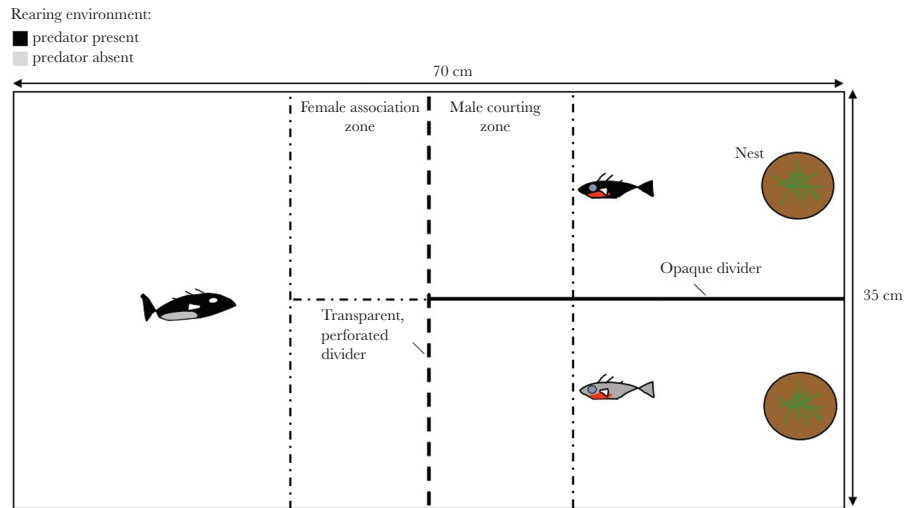
Experimental set-up

Experiments took place between 29 August and 17 September 2010. At that time fish were approx. one year old. At the start of the experiment, we counted all males that showed signs of developing nuptial coloration. Numbers did not differ significantly between the control and predator treatment (Wilcoxon matched-pairs signed-rank test, $N = 8$ families, $W = 15.0$, $P = 0.4$). The males were subsequently removed from the group tank and isolated in 22 × 25 × 48 cm plastic tanks, filled to a level of 15 cm with aerated water from the home-tank treatment. The tanks were equipped with a Petri dish filled with sand and 1 g of green wool cut in small pieces as nesting material. We stimulated males to build a nest by presenting a randomly chosen gravid female from the stock population held in a net cage for 5 min every day (Frommen and Bakker 2006). During this time, water was exchanged daily as described above and replaced by either perch-conditioned water or aged water. As soon as nests were completed in the Petri dish and males were courting the stimulus females vigorously, they were considered ready for the experimental trials.

To assess female preferences during mate choice, we employed a dichotomous set-up and quantified the time females allocated towards two reproductively active males reared in different treatments (Figure 1). The experimental tank (70 × 35 × 40 cm) was divided transversally into two equal-sized compartments (35 × 35 × 40 cm) by a transparent plastic partition with holes to allow water circulation. The partition was temporarily covered with an opaque, black plastic divider that was lifted at the beginning of the trial (Figure 1). One of the two compartments was further divided longitudinally by an opaque divider, creating the two male compartments. The undivided compartment constituted the female section and contained a plastic plant in the center to offer refuge and facilitate acclimatization. The whole apparatus was wrapped with a dark plastic foil and placed behind a black curtain to prevent disturbance during trials. We positioned a webcam above each tank to record fish behavior. A neon lamp placed 60 cm above the water level in the center of each tank ensured uniform illumination.

Experimental procedure

Two males and two females coming from both rearing conditions formed one experimental unit. Each male dyad was tested four times, and each female was tested twice. Between experimental units, treatment order and the positions of individuals in the tanks were randomized.

We tested the preference of females (either reared under simulated predator presence or absence) in the presence of two simultaneously presented males (one raised in presence of predators and one in absence). The testing of a given unit lasted for two days. To examine the effect of acute predator presence on mating behavior, we tested each female twice with the same stimulus males, once in perch-conditioned water and once in aged water. On the

Figure 1

The experimental set-up viewed from above. In the three-compartment tank, a female (left compartment) was given a choice between two stimulus males: one reared under chronic predator presence (upper right compartment) and the other one raised in the absence of predator cues (lower right compartment). Focal females were also either reared under chronic predator presence (in the present case) or in a predator-free environment. The same experimental triad (i.e., the focal female and the two stimulus males) were tested twice, once under acute predator presence (i.e., in aged water conditioned with predator cues) and once without acute predator presence (i.e., in aged water without further cues). The two males were then presented to a further female from the other development environment. We estimated female mating preferences for the respective males by measuring the time females spent in the association zones in front of the males. Male courtship activity was assessed by measuring the time males spent in the courtship zones. Zone boundaries were drawn on the bottom of the tank with a black marker.

first day, we used one of the two females along with two males. On the second day, the same two males were used again but exposed to the female originating from the other development environment. The experiment consisted of 19 units, that is, we tested 19 females reared under chronic predator presence and 19 females reared in aged water without any predator cues. Males and females within experimental units were unrelated to each other to avoid relatedness affecting mating decisions.

On the day of the respective mate choice trials, we filled all compartments of the experimental tanks either with perch-conditioned water or with aged water to a level of 13 cm. Next, we moved the two males (one male from each environment) together with their nests into the experimental tank. Males were matched in SL to the nearest 2 mm within pairs. They readily re-accepted their nests after the movement as indicated by nest repair behavior and showfanning (Rick and Bakker 2008b; Mehlis et al. 2009). After a 30-min acclimatization period, we added a gravid focal female originating either from the predator-exposed development treatment or from the control group to the female compartment. Females ready to spawn can easily be recognized by their swollen abdomen and by eggs visible through the skin near the cloaca (Frommen et al. 2012).

After 5 min, we lifted the opaque divider, allowing the female to evaluate both males. Once the female had inspected both males, as indicated by entering the respective choice zone in front of each male, we recorded the behavior of all individuals for 15 min. At the end of the trial, we lowered the divider. Two minutes later, we removed the males and immediately photographed them in a water-filled photo box (10 × 5 × 5.5 cm) under standardized light conditions in front of a uniform black background and illuminated with a Volpi Intralux 6000 fibre optic light source (see Bakker and Mundwiler 1994; Frommen et al. 2008 for details). We took photographs using a Canon Eos 400D Camera equipped with an EFS 18–50 mm lens. To correct for potential illumination differences between pictures, the standardized white side of a Novoflex Zebra

Grey Card was visible on each image (Bakker and Mundwiler 1994). We took photos of the ventral and the left lateral side of males.

We performed the same behavioral test with the same three individuals in a second experimental tank filled with the opposite treatment water, following the same protocol as before. Immediately after the pictures were taken, males and their nests were placed in the second experimental tank. To control for any side bias, we inverted the positions of the two males. All males showed normal swimming and nest repair behavior within few minutes. After the trial, we photographed males again and measured the SL and mass (M) of both males and females. Based on these measurements, we calculated the body condition (BC) for each male as BC = 100*M/SL³ following Bolger and Connolly (1989). Subsequently, we placed males and their nests back into their individual tanks. To ensure that females were indeed ready to spawn they were placed in a tank of a nest-tending male that did not take part in the experiment (Frommen and Bakker 2006). All females spawned with that male within 24 h after termination of their trial. On the following day, the same two males were tested together again following the experimental protocol described above, but with a female reared in the other treatment condition. As the order of the acute exposure treatments was inverted, we could examine their effects on male ornamental coloration. We changed the water in the experimental tanks after each trial.

Video and photo analyses

From each photo, we measured male coloration at ten standardized points on the throat and six points on the eye following the protocol described in Frommen et al. (2008). We measured coloration with Photoshop in the CIE (L*, a*, b*) color space, which has the advantage of being more device-independent than the RGB color space (Stevens and Cuthill 2005). The "a*" axis represents the color spectrum ranging from green (negative values) to magenta (positive

values), and the "b*" axis represents the range from blue (negative values) to yellow (positive values). Before measurement, we corrected photographs for any differences in brightness (L*) using the Novoflex Zebra Grey Card as a white standard (Bakker and Mundwiler 1994). We took measurements at the predefined spots measuring 5 x 5 pixels with the color sampler tool CS3 in Adobe Photoshop. Three indices per picture were calculated by averaging the ten values of a* and b* for the throat and the six values of b* for the eye, called redness A, redness B, and blueness, respectively. Because average estimates of redness A and B were correlated (Pearson's $r = 0.37$, $N = 76$, $P = 0.001$), we combined these two variables via a PCA. The first component explained 68.36 % of the variance, and both original variables loaded strongly and positively on it (both $r = 0.83$). We used component scores on PC1 as overall measures of redness in further analyses.

We analyzed videos blindly with respect to fish identity, the chronic treatment fish were taken from, and the level of predation risk. For video analysis, we used the "Observer" software by Noldus (Wageningen, Netherlands). We measured the time females spent in the choice zone in front of the respective male compartments (Figure 1). Time spent close to a male stimulus has been shown to reliably predict mating probability in this species (McLennan and McPhail 1990; Milinski et al. 2005). We furthermore measured male courtship activity. Studies on free-ranging male three-spined stickleback often use the number or duration of zig-zag courtship dances to measure male sexual activity (e.g., Kraak and Bakker 1998; McGhee et al. 2015; Head et al. 2017). However, these dances are performed over considerable distances. As our set-up restricted male movements to a maximum distance of 35 cm (the total length of male compartments), males remained courting close to the clear divider and were highly active during trials. Previous studies showed that under such conditions, time being close to a gravid female is in strong positive correlation with the amount of zig-zag dances (Rowland et al. 1991; Kraak and Bakker 1998). Therefore, we used the time the respective male spent in a given courtship zone (11.5 × 11.5 cm, Figure 1) bordering on the female compartment as a proxy for male courtship activity (c.f. Rowland et al. 1991; Kraak and Bakker 1998; Rick and Bakker 2008b; Frommen et al. 2009b). We judged experimental fish to have entered a zone once their head and pectoral fins crossed the zone boundary.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 25. We analyzed the effects of chronic predator presence on male SL and BC using linear mixed models (LMM). We log-transformed SL data to meet the assumptions of homogeneity of variances and normality of model residuals. We entered SL or BC as the dependent variable, chronic predator presence as a fixed factor, and family as a random factor. Because we measured SL and BC of males twice (see above), we calculated averages for SL and BC to simplify the analysis and avoid pseudo-replication. To examine whether chronic predator presence during development or acute predator presence during mate choice trials affected male coloration (eye blueness and throat redness) or behavior (time spent close to the female), we ran repeated measures linear mixed models (rmLMM) separately for each dependent variable. Throat redness, eye blueness, or time spent close to the female were the dependent variables, chronic predator presence and acute predator presence (present/absent) were entered as fixed effects and male ID as a repeated-measures random variable. We also entered the interaction between chronic

and acute predator presence into the model. To account for the use of more than one male per family, we entered family of males as a random factor.

We analyzed the effects of the males' and females' developmental environment, acute predator presence, and male traits on female mating preference using LMMs. We entered the time females spent close to a male as the dependent variable, male and female developmental environment (chronic predator cues present/absent), and acute predator cues (present/absent) as fixed effects, and trial ID as a repeated-measures random variable. To assess how male phenotypic traits affect female preference, we entered residual values of male SL, BC, eye blueness, throat redness, and the time males spent close to the female as covariates. We used residual values to account for male traits' potential dependence on chronic and acute predator presence, thereby avoiding interdependence among explanatory variables. Residual values were standardized scores of male phenotypic traits calculated separately for the four combinations of the male development environment and acute predator presence during choice trials. Because males and females were used repeatedly within a given experimental unit, we also entered male ID and female ID as random effects to avoid pseudo-replication. We entered the three two-way interactions among the fixed effects into the model. We applied backward stepwise removal procedures (Grafen and Hails 2002) for model simplification. In brief, we removed non-significant terms one by one, starting with the least significant interactions, followed by the main effects until only significant terms (and non-significant ones constituting a significant interaction) remained in the final model. Removed variables were re-entered one by one to the final model to obtain relevant statistics. We retained random effects in LMMs to avoid pseudo-replication and the resulting inflation of the degrees of freedom. To avoid inflation of the Type I error rate, we applied Bonferroni-correction on P-values obtained in post hoc tests. We confirmed that the fitted models fulfilled requirements by plot diagnosis. All tests were two-tailed, with α set to 0.05.
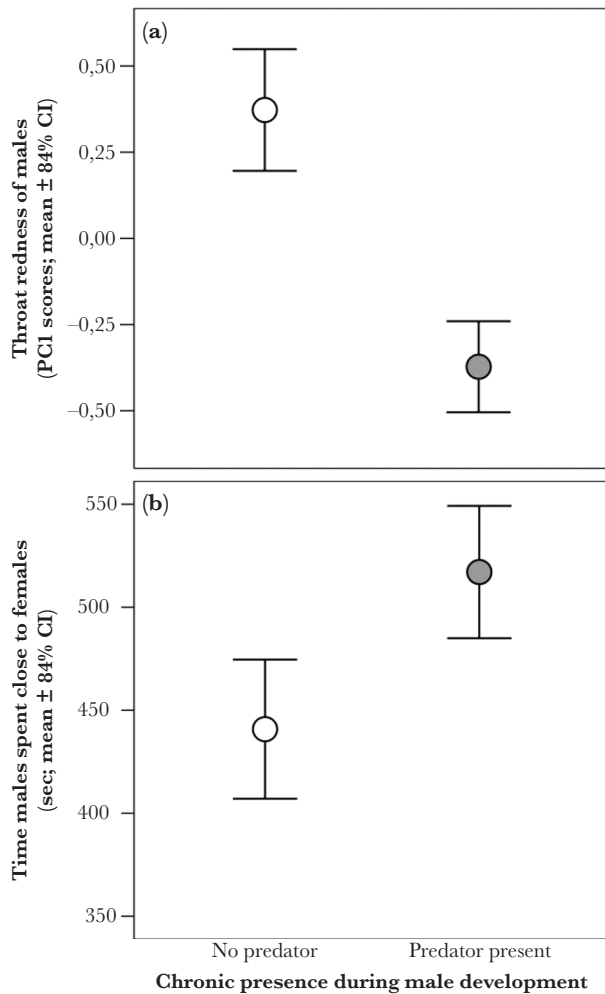
RESULTS

Effects of chronic and acute predator presence on male traits

Chronic exposure to chemical cues of predators affected the development of secondary sexual traits and courtship behavior of males: males reared in the absence of chronic predator presence had higher values of throat redness (rmLMM; $F_{1,70.1} = 4.12$, $P = 0.046$; Figure 2a) and spent less time close to the female ($F_{1,66.1} = 5.72$, $P = 0.02$; Figure 2b) than those reared in the presence of predators, whereas a similar effect on eye blueness was not apparent ($F_{1,71.5} = 2.63$, $P = 0.11$). Acute predator presence or its interaction with chronic predator presence did not affect male ornamentation nor male behavior (acute risk: all $P > 0.58$; acute presence × chronic presence: all $P > 0.38$). Males in the two chronic exposure treatments did not differ in SL or BC (LMM; SL: $F_{1,35.4} = 0.06$, $P = 0.81$; BC: $F_{2,36} = 0.38$, $P = 0.54$).

Effects of chronic and acute predator presence and male traits on female mating preference

Female preference was affected by the interaction between chronic predator presence experienced by males during development and acute predator presence (LMM; $F_{1,68.8} = 14.65$, $P < 0.001$): in mate choice trials performed under acute predator presence, females did not discriminate between males coming from the two rearing
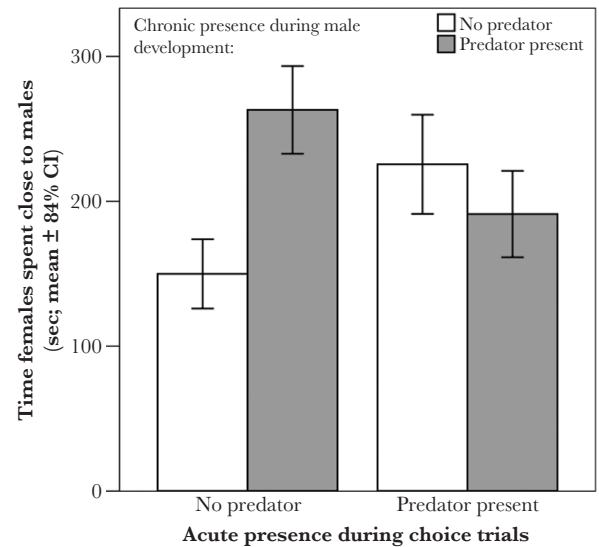
Figure 2
(a) Throat redness of males and (b) the time males spent close to the female in relation to the development environment of males (chronic absence or presence of predators). For the ease of interpretation, means of raw data ±84% CI are shown.

Figure 3
Female preferences measured as the time females spent close to males reared in different environments (chronic absence or presence of predators) under different experimental conditions (acute absence or presence of predators). For the ease of interpretation, means of raw data ±84% CI are shown.

presence experienced during development (from the egg stage to reaching sexual maturity) affected male ornamental traits and courtship behavior whereas acute predator presence experienced during mate choice affected female mating preferences, depending on the male's predatory history.

Chronic exposure to chemical cues of predators resulted in a weaker expression of red throat coloration in males and in an increase in courtship activity compared with males that developed in the absence of predator cues. Plastic responses to predator presence can result in more cryptic nuptial coloration in prey species (Ruell et al. 2013; Winandy and Denoel 2015; Meuthen et al. 2018). The bright red coloration of stickleback's throat has been shown to increase the risk of predator-attacks (Maitland 1965; Moodie 1972; Johnson and Candolin 2017). Hence, a reduction in the red ornamentation's conspicuousness is likely to result in lowered predation risk when predators are present in the environment. Enhanced activity in the presence of females may, on the other hand, only temporarily lead to an increased risk of detection by predators. At the same time, it may be necessary to attract females despite the less conspicuous coloration. This result is in accordance with life-history theory, predicting that individuals should take higher risks during reproduction, especially if their chance of reproducing again is low (Candolin 1998). Comparable to many other populations (Wootton 1976) the breeding season of three-spined stickleback on Texel lasts only a few weeks in early summer, and adults die afterward (Kemper 1995). Thus, opportunities to reproduce are limited to one short period, which should lead to an increased willingness to accept higher predation risk, especially during the phase of active courtship, which usually lasts only for a few minutes per mating (Tinbergen 1952). Indeed, reproductively active male and female three-spined stickleback are more risk-prone in the presence of a predator than non-reproductive ones (Frommen et al. 2009a). In contrast to throat color, eye color was not influenced by chronic predator exposure. This suggests that these different color signals are expressed independently from each other and may provide females with multiple cues to assess male quality (Frischknecht 1993; Flamarique et al. 2013).

regimes ($F_{1,129.8} = 0.76$, $P = 0.38$), whereas in the absence of predators during choice trials females spent more time close to males reared under chronic predator presence than to males reared in the absence of predator cues ($F_{1,123.8} = 16.33$, $P < 0.001$; Figure 3). Importantly, the developmental environment of females did not affect their preference ($F_{1,66.9} = 0.16$, $P = 0.69$). The interactions between the male and female developmental environments and the female developmental environment and acute predator presence were non-significant (both $P > 0.36$). Residual values of male SL and eye blueness positively affected the time females spent close to a male (LMM; SL: $F_{1,67.8} = 10.28$, $B = 1.23$, $SE = 0.38$, $P = 0.002$; eye blueness: $F_{1,94.9} = 7.71$, $B = 1.02$, $SE = 0.37$, $P = 0.007$). However, female preference was not affected by the residual values of the time males spent close to the divider ($F_{1,132.1} = 0.11$, $P = 0.74$), male throat redness ($F_{1,76.1} = 0.37$, $P = 0.55$) or male BC ($F_{1,67} = 0.83$, $P = 0.36$).

DISCUSSION

In the present study, we show that the presence of olfactory predator cues has a complex impact on male traits and female preferences that play central roles in sexual selection. Chronic predator

Unlike male traits, female preference was not affected by chronic predation risk experienced during development. Furthermore, in contrast to previous studies conducted under predator-free conditions (e.g., Bakker and Milinski 1993; Candolin 1997; Rowland 1995), neither male's throat coloration nor courtship intensity influenced female preference. This might be explained by changes in female preference for male color and activity depending on acute predation risk (Candolin 1997). Indeed, several studies on female preferences for male nuptial coloration and courtship activity showed that under certain circumstances, the red coloration (McDonald et al. 1995; Candolin et al. 2007; Engström-Öst and Candolin 2007) and courtship activity (Rowland 1995; Künzler and Bakker 2001) can lose its significance for mate choice, whereas other indicators of quality can gain importance instead (Boughman 2001; Rick and Bakker 2008a; Heuschele et al. 2009; Flamarique et al. 2013). In line with this argument, acute predator presence during choice trials and male developmental environment interactively influenced female preference, indicating that preferences may shift when acute predator presence changes. These results are in accordance with recent studies on three-spined stickleback suggesting that female and male mate choice is affected by the presence of predators (Kozak and Boughman 2015; McGhee et al. 2015), and highlight that incorporating natural threat stimuli can change the outcome of studies on sexual selection and mate choice. Therefore, our results call for the application of more natural settings and planned experimental co-testing of potentially important ecological factors when investigating sexual preferences.

In our experiment, males with more intensely colored blue eyes and larger SL were preferred over duller-colored and smaller males (cf., Rowland 1989; Flamarique et al. 2013), and this was independent of acute predator presence. Interestingly, male traits that appeared responsive to chronic predator presence (i.e., throat redness and male courtship behavior) were less important for female choice in our study. In contrast, male traits that were less affected by chronic predation threat (i.e., eye blueness) or where variation was kept minimal per experimental design (SL) seemed to be highly relevant for female choice, independent of predators' acute presence. These findings may help explain the maintenance of variation in sexually selected traits, leaving different male traits free to respond to sexual selection depending on the chronic and acute predator presence.

Several studies on predator recognition have found that fishes and other animals get habituated to the simulated presence of predators, especially when predator cues are presented over a prolonged period (e.g., Brown et al. 2006; Raderschall et al. 2011; Imre et al. 2016). Habituation might hence be an interesting alternative explanation for our results. Indeed, it is straightforward to imagine that individuals that face olfactory stimuli of perch during their whole life without ever getting attacked would show bolder behavior under acute risk than fish that experience such cues for the first time. However, the male stickleback in our study did not show any differences between the two acute set-ups, suggesting that habituation effects do not impair their courtship behavior or expression of red throat coloration. Similarly, long-term exposure to predator cues did not lead to differences in the choice of females coming from the different developmental treatments. Females from both treatments showed no clear preferences when the acute presence of predators was simulated, while they expressed similar preferences under predator-free conditions. Elucidating the general role of habituation to predator cues and the question of how cues presented in different modalities or coming from different predators influence plastic and acute responses of stickleback pose interesting

questions for future studies (e.g., Chivers et al. 2016; Fischer et al. 2017; Fischer and Frommen 2019).

In conclusion, we show that chronic and acute predator presence can influence the expression of sexually selected traits in three-spined stickleback. Such phenotypic plastic effects may contribute to speciation processes if male sexual ornaments and female choice develop in the same direction (i.e., females prefer male phenotypes expressed in the same predator-environment as themselves). However, in our study population, plastic antipredator responses in male sexual ornaments and female preferences did not align with each other. Hence, the interactive effect of chronic and acute predator presence on male sexual ornaments and female mating preference may lead to dynamic processes that can alter the strength and even the direction of sexual selection, resulting in temporal and spatial variation in secondary sexual traits.

ETHICAL STATEMENT

The experiment followed the ASAB/ABS guidelines for the treatment of animals in behavioral research and teaching. The experiments were discussed and approved by the institutional ethics committee in accordance with Good Scientific Practice guidelines and national legislation. According to the legislation of Austria no further ethical permits were required at the time the experiments were conducted.

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AUTHOR CONTRIBUTIONS

J.G.F., V.B., and A.H. conceived the study. J.G.F. and A.H. organized funding. J.G.F. and V.B. set up the breeding line. F.S. conducted the experiment and analyzed the videos. A.H., T.T., and F.S. ran the statistical analyses. T.T. wrote the first draft of the manuscript, with support from J.G.F. and A.H. All authors edited the manuscript and approved its final version.

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Data availability: Analyses reported in this article can be reproduced using the data provided by Frommen et al., (2021).

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