The Company of
Biologists

REVIEW

Diversity in reproductive seasonality in the three-spined stickleback, *Gasterosteus aculeatus*

Asano Ishikawa[1,2,*] and Jun Kitano[1,2]

ABSTRACT

The annual timing of reproduction is a key life history trait with a large effect on fitness. Populations often vary in the timing and duration of reproduction to adapt to different seasonality of ecological and environmental variables between habitats. However, little is known about the molecular genetic mechanisms underlying interpopulation variation in reproductive seasonality. Here, we demonstrate that the three-spined stickleback (*Gasterosteus aculeatus*) is a good model for molecular genetic analysis of variations in reproductive seasonality. We first compiled data on reproductive seasons of diverse ecotypes, covering marine-anadromous, lake and stream ecotypes, of three-spined stickleback inhabiting a wide range of latitudes. Our analysis showed that both ecotype and latitude significantly contribute to variation in reproductive seasons. Stream ecotypes tend to start breeding earlier and end later than other ecotypes. Populations from lower latitudes tend to start breeding earlier than those from higher latitudes in all three ecotypes. Additionally, stream ecotypes tend to have extended breeding seasons at lower latitudes than at higher latitudes, leading to nearly year-round reproduction in the most southern stream populations. A review of recent progress in our understanding of the physiological mechanisms underlying seasonal reproduction in the three-spined stickleback indicates that photoperiod is an important external cue that stimulates and/or suppresses reproduction in this species. Taking advantage of genomic tools available for this species, the three-spined stickleback will be a good model to investigate what kinds of genes and mutations underlie variations in the physiological signalling pathways that regulate reproduction in response to photoperiod.

KEY WORDS: Seasonal reproduction, Photoperiodism, Life history trait, Interpopulation variation

Introduction

The timing of reproduction is one of the most important life history traits determining fitness (Roff, 2002; Stearns, 1992). During reproduction, organisms invest in developing reproductive organs, exhibiting secondary sexual characters, seeking mating partners, competing with rivals of the same sex or taking care of progeny. Because reproduction is costly, natural selection drives organisms to breed only during optimal seasons of the year when they can maximise their reproductive success and the survival of their offspring. For example, optimal times for breeding may occur when food is abundant, predators are scarce or environmental conditions, such as temperature and precipitation, are optimal for offspring growth and survival. Because the seasonal patterns of these ecological and environmental factors differ between populations, the optimal timing of reproduction is also expected to differ.

Variation in reproductive seasonality is prevalent across the animal kingdom. Because seasonality differs between latitudes, latitudinal variation in the timing of reproduction has been widely observed not only between species but also within species. For example, in birds, breeding periods tend to be concentrated into spring or early summer and begin later in the year with increasing distance from the equator, while a peak reproductive season is less pronounced because breeding starts earlier and ends later in regions near the equator (Baker, 1939). This difference may reflect the degree of seasonality at a given latitude which determines the optimal conditions for breeding. Similarly, for example, in deer mice (*Peromyscus* spp.), northern populations possess very short and discrete breeding seasons, while southern populations breed continuously (Bronson, 1985). Even within the same latitude, populations exploiting different niches often differ in the timing of reproduction, which can contribute to reproductive isolation between sympatric and parapatric ecotypes (Coyne and Orr, 2004; Mayr, 1963; Nosil, 2012). For example, migratory and resident ecotypes of three-spined stickleback (*Gasterosteus aculeatus* species complex) inhabiting the same river differ in breeding season with little overlap (Hagen, 1967; also see below).

The physiological mechanisms that control seasonal reproduction have been investigated in diverse taxa. A wide variety of organisms use day length to predict seasonal changes, as this provides a highly reliable and predictable seasonal cue (Bradshaw and Holzapfel, 2007). Light is sensed by the eyes and/or deep brain photoreceptors, whose signals are transmitted to the brain regions that regulate the hypothalamic–pituitary–gonadal (HPG) axis. Temperature and precipitation also inform organisms of the seasons. Despite extensive studies on the physiology of photoperiodism and the ecology of optimal timing of reproduction, we know little about what genes and mutations underlie the diversity of seasonal reproduction.

The three-spined stickleback represents a good model to investigate the molecular and genetic mechanisms underlying variation in reproductive seasonality (Fig. 1). First, this species inhabits diverse aquatic environments from marine to freshwater across a wide range of latitudes in the northern hemisphere (Bell and Foster, 1994; Wootton, 1976, 1984). Variations in seasonal reproduction have been observed between ecotypes and between latitudes, which we review below (Figs 1 and 2) (Bertin, 1925; Hagen, 1967; Ishikawa and Kitano, 2019; Yeates-Burghart et al., 2009). The evolution of a similar subset of phenotypically and ecologically divergent ecotypes in multiple different lineages enables us to investigate convergent evolution. Second, genetic and genomic tools are available for the three-spined stickleback,

[1]Ecological Genetics Laboratory, National Institute of Genetics, Yata 1111, Mishima, Shizuoka 411-8540, Japan. [2]Department of Genetics, Graduate University for Advanced Studies (SOKENDAI), Yata 1111, Mishima, Shizuoka 411-8540, Japan.

*Author for correspondence (asishika@nig.ac.jp)
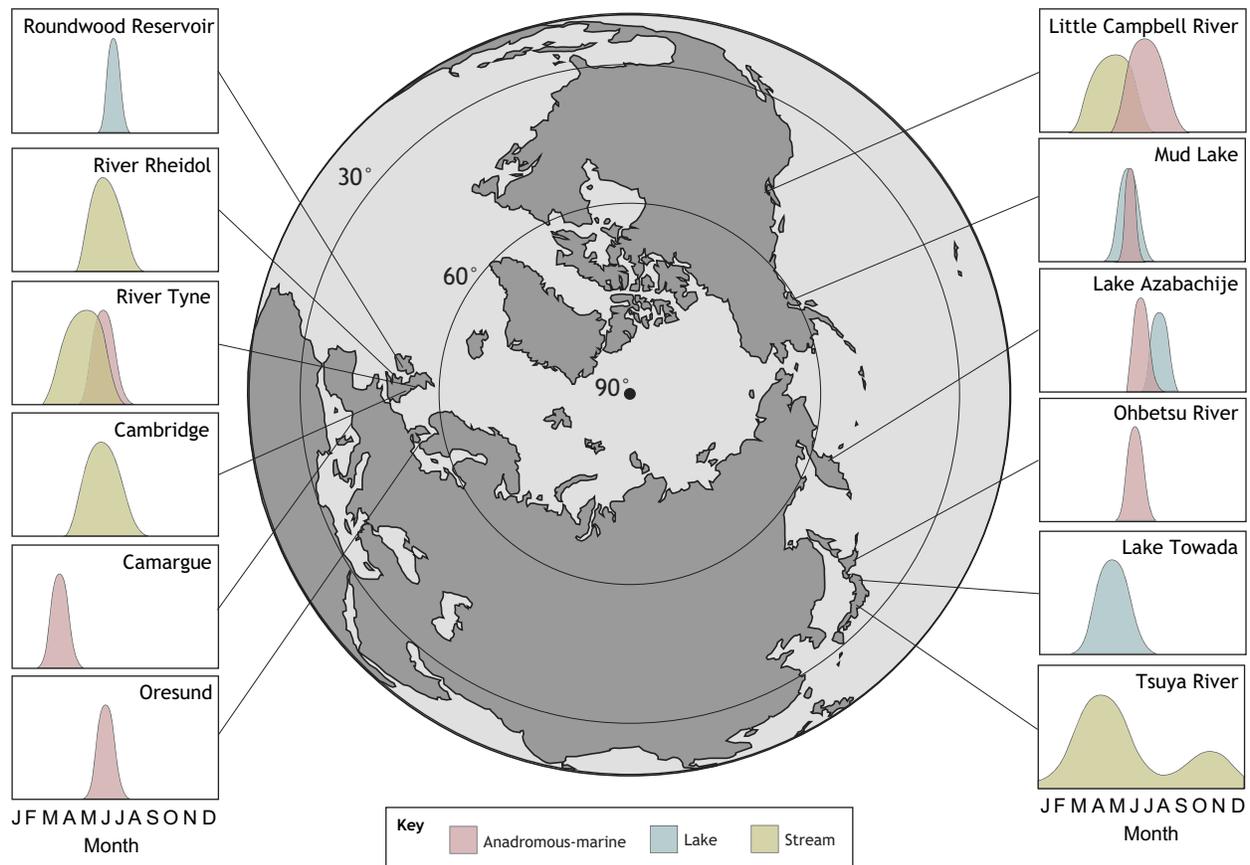
A.I., 0000-0003-1628-8339; J.K., 0000-0001-8659-5698

Fig. 1. Observed diversity in breeding seasons in several populations of the three-spined stickleback, *Gasterosteus aculeatus*. In the plots, the *x*-axis indicates the time of year from January (J) to December (D). These data were extracted from the following references: Borg (1982b); Craig-Bennett (1931); Crivelli and Britton (1987); Dauod et al. (1985); Hagen (1967); Jones et al. (2006); Karve et al. (2008); Kume et al. (2005); Mori (1985); Sokołowska and Kulczykowska (2006); Wootton (1984); Ziuganov et al. (1987); and S. Mori, personal communication.

such as a linkage map, a reference genome sequence and genetic engineering. Recent genetic and genomic studies have revealed the molecular mechanisms underlying the morphological, physiological and behavioural divergence between anadromous and freshwater populations of three-spined stickleback (Chan et al., 2010; Greenwood et al., 2013; Indjeian et al., 2016; Ishikawa et al., 2019; Marques et al., 2017; Miller et al., 2007; O'Brown et al., 2015; Xie et al., 2019). Third, as discussed in detail below, the endocrine mechanisms underlying seasonal reproduction have been investigated in the three-spined stickleback (Hellqvist et al., 2006; Shao et al., 2013, 2015; Sokołowska et al., 2004).

In this review, we first compile and analyse data on the breeding seasons of three-spined stickleback populations. Although Baker (1994) previously compiled some of these data, more information has become available during the last 25 years. Second, we review what is known about the neuroendocrine mechanisms underlying seasonal reproduction in the three-spined stickleback. Finally, we discuss future directions employing integrative physiological genomics towards a better understanding of the molecular genetic mechanisms responsible for variation in seasonal reproduction in sticklebacks.

Diversity of seasonal reproduction in sticklebacks
Literature data collection and analysis
To compile a comprehensive dataset on breeding seasons in three-spined stickleback populations, we used data collected on 146 populations from the published literature and unpublished

information. These data have been deposited in Dryad (https://doi.org/10.5061/dryad.pb7v936) (Ishikawa and Kitano, 2019). To investigate the effects of ecotype, we categorized the populations into anadromous-marine, lake and stream ecotypes. In several previous studies, anadromous and marine ecotypes have been categorized separately (Baker, 1994), but it is often difficult to distinguish them clearly, because there is a range of salinities from freshwater to seawater at the breeding sites and also a range of migration distances after moving into rivers and lakes (Kitano et al., 2012). To analyse the effects of latitude and day length on the start and end of the spawning season, we used the latitude and longitude of each habitat, whenever available, and calculated the day length using the online application SunCalc (https://www.suncalc.org). We conducted a generalised linear model analysis in order to test whether ecotype and latitude influence the duration, start and end of the reproductive period. As three-spined sticklebacks are genetically separated between Atlantic and Pacific lineages, we added the lineage as a covariate (Fang et al., 2018; Jones et al., 2012). *P*-values were obtained using a chi-squared test to compare two models with the test predictor and without the predictor. A Tukey's *post hoc* test was conducted using the lsmeans package in R (Lenth, 2016). To test the association between latitude and the duration, start and end of the reproductive period, Pearson's correlation test was conducted using R version 3.3.1. Breeding seasons were defined as periods when either of the following were observed in the spawning sites: breeding colour, nesting behaviours, spawning behaviours, mature gonads in adult fish or newly hatched larvae (Baskin, 1974). It
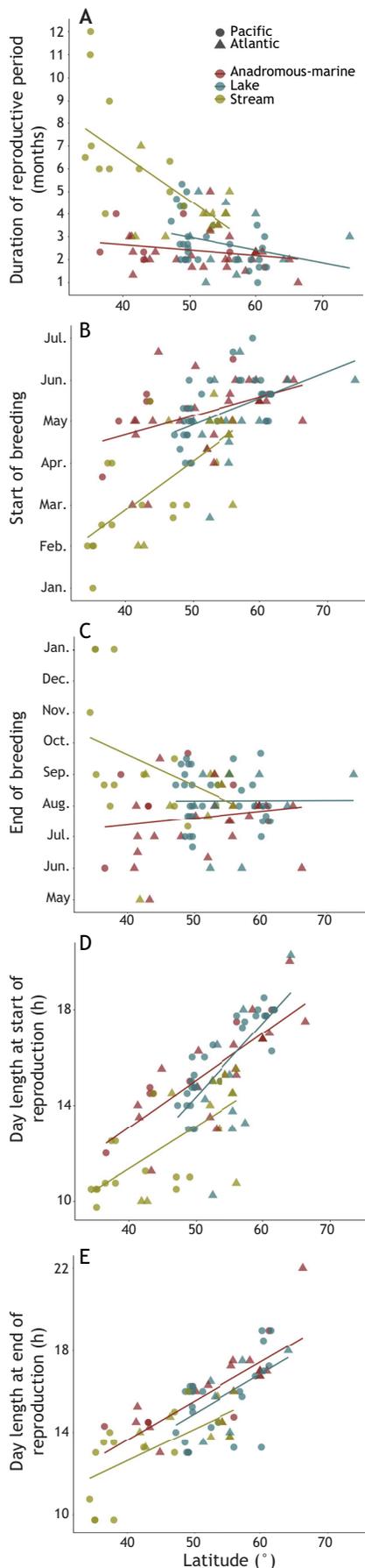
Fig. 2. Effect of ecotype and latitude on reproductive seasonality. Relationship of latitude with (A) duration of the reproductive period, (B) timing of the initiation of spawning, (C) timing of the end of spawning, (D) day length at the start of spawning and (E) day length at the end of spawning. Day length was calculated using the online application SunCalc (https://www.suncalc.org).

should be noted that phylogenetic correction was not made, so future studies taking phylogeny into account should be conducted to obtain more robust results, although clear resolution of phylogenetic relationships of populations within species is often difficult.

Diversity in the duration of the reproductive period

The duration of the reproductive period varied significantly depending on both the ecotype and the latitude (ecotype: $P=1.57\times10^{-13}$, latitude: $P<2.0\times10^{-16}$, lineage: $P>0.05$) (Figs 1 and 2). Anadromous-marine ecotypes had shorter breeding seasons than stream ecotypes (mean±s.d. 2.37±0.82 versus 5.42 ±2.37 months, Tukey's *post hoc* test: $P<0.01$), with less variation and no latitudinal trends (Pearson's correlation: $r=-0.24$, $P=0.18$) (Fig. 2A). For instance, the anadromous-marine ecotypes even from lower latitudes, such as the Camargue region of southern France (approximately 43°N), had short breeding seasons comparable to those of anadromous-marine populations from higher latitudes. In contrast, stream ecotypes had breeding seasons that were significantly associated with latitude (Pearson's correlation; $r=-0.68$, $P<0.01$). In particular, stream populations from lower latitudes showed longer breeding seasons than those from higher latitudes. In several stream habitats, almost year-round reproduction has been reported (Baskin, 1974; Mori, 1985). For example, in the Tsuya River in the mainland of Japan (approximately 35°N), stream sticklebacks reproduce throughout the year, with bimodal peaks of breeding activity in spring and autumn (Mori, 1985) (Fig. 1). A stream ecotype in the Santa Clara River of southern California, USA (approximately 34°N), showed similar year-round reproduction with a large peak of spawning activity in spring and a small peak in autumn (Baskin, 1974), suggesting convergent evolution of year-round reproduction with bimodal peaks in low-latitudinal regions. In contrast, stream populations from middle latitudes, such as Scotland (approximately 55°N), reproduce for only 3.5–4.0 months in spring, similar to marine and lake populations from the same latitude. Lake ecotypes showed reproductive period durations (mean ±s.d. 2.74±1.13 months) intermediate between those of marine and stream ecotypes with latitudinal trends, although this latitudinal trend was not significant (Pearson's correlation: $r=-0.29$, $P=0.059$). Several lake ecotypes from high latitudes, such as Johnson Lake in Alaska, USA (approximately 60°N), reproduced only for 1 month, which is the shortest reproductive period ever reported, together with the anadromous-marine populations in the White Sea (Allen and Wootton, 1984; Engel, 1971; Golovin et al., 2019; Ishikawa and Kitano, 2019; Ivanova et al., 2019; Wootton et al., 1978). There are few reports regarding the reproductive period of lake populations from latitudes lower than 50°N because lake ecotypes are relatively uncommon in these regions (Fig. 2).

Start of reproduction

The prolonged reproduction in stream and lake populations was partially explained by the early onset of breeding (ecotype: $P=2.67\times10^{-8}$, latitude: $P<2.0\times10^{-16}$, lineage: $P>0.05$) (Fig. 2B). Populations from lower latitudes typically initiated reproduction earlier in the year than those from higher latitudes (Pearson's correlation: $r=0.63$, $P<0.01$). Especially in lower latitudes, stream

populations began spawning much earlier than anadromous-marine and lake populations, resulting in higher latitudinal dependence in stream populations (Pearson's correlation: $r=0.70$, $P<0.01$) than in anadromous-marine (Pearson's correlation: $r=0.45$, $P<0.01$) or lake populations (Pearson's correlation: $r=0.46$, $P<0.01$). Temporal differences in breeding season partially contribute to prezygotic reproductive isolation between sympatric ecotypes. For example, anadromous-marine sticklebacks begin spawning later than sympatric stream populations in several localities (Hagen, 1967; Jones et al., 2006). In contrast, in Lake Azabachije on the Kamchatka Peninsula of Russia, a freshwater population was reported to initiate reproduction later than an anadromous population (Ziuganov et al., 1987) (Fig. 1).

Analysis of day length at the onset of spawning in each habitat revealed that it varied depending on both ecotype and latitude (ecotype: $P=1.24\times10^{-5}$, latitude: $P<2.0\times10^{-16}$, lineage: $P=0.0013$). Stream ecotypes started to breed under shorter day length than anadromous-marine or lake ecotypes (Tukey's post hoc test: $P<0.01$) (Fig. 2D). More than half of the stream populations initiated breeding under a day length of 12 h or less, whereas most of anadromous-marine and lake populations did not begin spawning until the day length reached 12 h or more. In all ecotypes, there were positive relationships between day length at the onset of breeding and the latitude that the population inhabits (anadromous-marine: $r=0.81$, $P<0.01$, lake: $r=0.74$, $P<0.01$, stream: $r=0.69$, $P<0.01$).

Termination of reproduction

Late ending of the breeding season was another factor contributing to the prolongation of reproductive periods in the stream populations (ecotype: $P=0.00056$, latitude: $P=0.0083$, lineage: $P=0.0078$) (Fig. 2C). Stream populations in lower latitudes stopped spawning later than those at higher latitudes (Pearson's correlation: $r=-0.43$, $P<0.05$), but there was no latitudinal dependence among the anadromous-marine (Pearson's correlation: $r=0.18$, $P>0.05$) or lake populations (Pearson's correlation: $r=-0.006$ $P>0.05$). Similar to day length at the start of reproduction, both ecotype and latitude influenced day length at the end of spawning (ecotype: $P=0.042$, latitude: $P<2.0\times10^{-16}$, lineage: $P>0.05$), while there was no significant difference between ecotypes in any pairwise comparison using a post hoc test (Fig. 2D). In addition, there were positive relationships between day length at the end of breeding and latitude (anadromous-marine: $r=0.78$, $P<0.01$, lake: $r=0.56$, $P<0.01$, stream: $r=0.67$, $P<0.01$).

Physiological basis for seasonal reproduction in sticklebacks

Annual reproductive cycle of the three-spined stickleback

The annual reproductive cycle of the three-spined stickleback has been documented in a few marine and freshwater populations from regions with latitudes of 50–60°N under both natural and laboratory conditions (Borg, 1982b; Borg and Veen, 1982; Craig-Bennett, 1931; Sokołowska and Kulczykowska, 2006). In these populations, the main processes of gonadal development occur several months before the onset of spawning. In males, spermatogenesis starts at the end of breeding in summer and is completed by late autumn or early winter. In females, oogenesis starts at the end of summer. Vitellogenesis, the deposition of large amounts of yolk in oocytes, starts during winter to early spring, and oocyte diameter increases significantly during this period. In spring, secondary sexual characters develop just before the spawning season. One of the most studied secondary sexual characters in sticklebacks is kidney hypertrophy in males. Male sticklebacks build a nest with

plant fragments using mucous glue substances secreted by the kidneys. As we discuss below, kidney hypertrophy is androgen dependent. In addition to kidney hypertrophy, male sticklebacks show breeding colour. In the majority of populations, the throat and ventral side of male sticklebacks become red during the breeding season (Tinbergen, 1951; Wootton, 1984). Female sticklebacks prefer to mate with more intensely red-coloured males (Bakker and Mundwiler, 1994; Milinski and Bakker, 1990). This red nuptial colour is due to the presence of red carotenoid pigments in the erythrophores. Astaxanthin, lutein and tunaxanthin have been identified in the skin of male sticklebacks during the breeding season (Wedekind et al., 1998). Redder males have more astaxanthin in their skin than yellowish males that possess more lutein/tunaxanthin. Male stickleback breeding colour is also androgen dependent (Wootton, 1976). Although the red breeding coloration is prevalent throughout the distribution range of the three-spined stickleback, black melanic breeding colour has been observed in several tannin-stained habitats (Bolnick et al., 2015; McPhail, 1969; Reimchen, 1989).

In addition to kidney hypertrophy and breeding coloration, the three-spined stickleback shows sexual dimorphism in many traits. Although there is some interpopulation variability, adult males generally have larger heads (Aguirre et al., 2008; Kitano et al., 2007; Leinonen et al., 2006, 2011; Spoljaric and Reimchen, 2008), larger mouths (Aguirre et al., 2008; Kitano et al., 2007; Leinonen et al., 2006, 2011; Spoljaric and Reimchen, 2008), larger brains (Kotrschal et al., 2012; Samuk et al., 2014), larger pectoral muscles (Bakker and Mundwiler, 1999; Hoffmann and Borg, 2006), more teeth (Caldecutt et al., 2001), a thicker epidermis (Burton, 1979) and faster time to peak jaw protrusion (McGee and Wainwright, 2013), and perform nest-building behaviours and courtship dances (Bell and Foster, 1994; Tinbergen, 1951; Wootton, 1976, 1984). However, there is little knowledge about when males and females begin to differ in these traits during the annual reproductive cycle.

Proximate environmental factors inducing seasonal reproduction

In the three-spined stickleback, the combined effect of photoperiod and temperature on sexual maturation and onset of breeding has been extensively investigated in marine populations from The Netherlands and the Baltic Sea (Baggerman, 1957, 1972, 1984; Borg, 1982a; Borg and Veen, 1982; Bornestaf and Borg, 2000). Photoperiod is a major environmental factor controlling the sexual maturation and development of secondary sexual traits (Baggerman, 1957, 1972, 1984; Borg, 1982a; Borg and Veen, 1982; Bornestaf and Borg, 2000). Long day lengths induce maturation, while short day lengths inhibit it, even if temperatures are high. Night interruption experiments, in which sticklebacks under a short photoperiod were exposed to a brief light exposure during the night, indicate that there is an endogenous daily rhythm of photosensitivity (Baggerman, 1984). However, another interpretation of these results is that the sticklebacks respond to the length of uninterrupted darkness (Borg, 2010).

Extraretinal photoreceptors can mediate photoperiodic effects on reproduction in the absence of eyes in many vertebrates, including sticklebacks (Borg, 1982a). Although photoreceptors are generally present only in the retina in mammals, other vertebrates have extraretinal photoreceptors such as those in the pineal organ. Both intact and blinded male sticklebacks matured under long-day conditions (16 h light:8 h dark) but did not mature under short-day conditions (8 h light:16 h dark) (Borg, 1982a). In fact, it has been demonstrated that the eyes are less effective than extraretinal

photoreceptors in stimulating sexual maturation in sticklebacks (Borg et al., 2004). When the tops of male stickleback heads were covered with opaque plastic foil and the eyes remained unaffected, less kidney hypertrophy was observed after exposure to a long photoperiod in winter compared with that in control fish with transparent foil covering the head. Similarly, female sticklebacks with covered heads had reduced levels of ovarian development and a lower proportion of them reached full maturation when compared with the controls. However, the significant inhibitory effect of head covering on long day length-dependent reproduction was observed only under long photoperiod at low light intensity but not under high light intensity. Under the high light intensity condition, both groups (head covered and non-covered groups) matured fully. These results suggest that extraretinal photoreceptors are more sensitive than retinal photoreceptors in mediating photoperiodic control of reproduction in the three-spined stickleback.

Several studies have demonstrated that temperature also plays a role in controlling sexual maturation in the three-spined stickleback (Baggerman, 1957; Borg, 1982a; Craig-Bennett, 1931; Lachance et al., 1987; Sokołowska and Kulczykowska, 2009). The effect of temperature differs between seasons. High water temperature stimulates gonad maturation and the development of secondary sexual characters in spring, but it diminishes sexual activity and accelerates the decline of secondary sexual characters at the end of the breeding season (Baggerman, 1957; Borg, 1982b; Borg and Veen, 1982; Sokołowska and Kulczykowska, 2009). In contrast, low temperature delays sexual maturation in males even under long-day conditions in spring, but inhibits the decline of reproductive activity at later stages of the breeding season (Borg, 1982b; Borg and Veen, 1982; Sokołowska and Kulczykowska, 2009). A previous study reported that temperature, but not photoperiod, influences the binding capacity of gonadotropin-releasing hormone (GnRH) in the pituitary, which displays a marked seasonal cycle with a high level during the breeding period, a low level during the refractory period and a non-detectable level in winter (Andersson et al., 1992). However, further studies are needed to understand how photoperiod, temperature and endogenous physiological factors interact and control the seasonal breeding cycle in sticklebacks.

Candidate hormones and genes involved in seasonal reproduction

Many hormones and genes are known to regulate seasonal reproduction in vertebrates. In vertebrates, the HPG axis plays a critical role in developmental regulation of the gonads and secondary sexual characters. GnRH is secreted in the brain from the hypothalamus. Gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), are produced by the anterior part of the pituitary gland and the sex steroids are produced by the gonads. Evolutionary change of this cascade is potentially involved in the diversification of seasonal reproduction among closely related species and populations.

Gonadal sex steroid hormones

Gonadal sex steroids play major roles in stimulating reproductive behaviours and the expression of secondary sexual traits in vertebrates. In sticklebacks, 11-ketotestosterone (11KT) is regarded as the most important androgen, as in other teleosts (Borg, 1994). Circulating levels of 11KT are at their highest level during the breeding season, when the male three-spined stickleback develops androgen-dependent secondary sexual traits such as kidney hypertrophy and breeding colour (Mayer et al., 1990). Kidney hypertrophy is inhibited by castration and can be induced by androgen treatment in both sexes (Borg, 1994;

Hoar, 1962). The androgens 11β-hydroxytestosterone (OHT) and 11-ketoandrostenedione (OA) were also highly effective in stimulating kidney hypertrophy as well as 11KT, but 5α-dihydrotestosterone (5αDHT) and testosterone were less effective (Borg et al., 1993). The levels of 11KT also change during the nesting cycle; they are much higher in nesting and courting males than in parenting and fanning males with fertilised eggs in the nests. Androgen treatment induces male nest-building behaviour in castrated males, indicating that it also controls male-specific reproductive behaviour (Hoar, 1962; Wai and Hoar, 1963). In contrast to secondary sexual characters, the correlation between plasma androgen levels and spermatogenesis is rarely found in fishes, including sticklebacks (Borg, 1994). In nesting males, spermatogenesis is inactive. In the post-breeding period, 11KT levels drop and androgen-dependent characters disappear, although spermatogenesis becomes active. Androgen treatment during the post-breeding period inhibits the onset of spermatogenesis but stimulates the expression of secondary sexual characters, indicating a possible negative correlation between spermatogenesis and androgens levels (Borg, 1981).

Females have much lower levels of 11KT than males, but they have significantly higher levels of 17β-oestradiol (E2) and similar levels of testosterone during the breeding season (Hellqvist et al., 2006; Kitano et al., 2011). Similar sex differences in levels of 11KT, testosterone and E2 are also found in other teleosts. Interpopulation variations in steroid hormone levels have been reported in three-spined sticklebacks. Females had higher levels of E2 in a limnetic population than in benthic or marine populations, and E2 levels were negatively correlated with interclutch interval (Graham et al., 2018). Because E2 drives vitellogenin production in the liver, higher E2 levels may increase the amount of vitellogenin for yolk production, allowing eggs to grow more quickly. Shorter spawning intervals may be favoured in limnetic populations, possibly leading to the evolution of higher E2 levels to support higher rates of egg production. In addition, female *Gasterosteus nipponicus*, which diverged from *G. aculeatus* between 0.68 and 1.5 million years ago (Ravinet et al., 2018), had significantly higher levels of E2 and testosterone than females of *G. aculeatus* during the breeding season (Kitano et al. 2011), suggesting that interspecies variation also exists in hormone levels.

Gonadotropins

Expression levels of gonadotropin show seasonal variation in three-spined stickleback. Vertebrates possess two gonadotropic hormones: FSH and LH. Both gonadotropins consist of two subunits, an α-subunit and a β-subunit; the former is shared among several pituitary glycoprotein hormones and the latter determines the specificity of receptor binding. Seasonal changes in *FSHβ* and *LHβ* expression have been reported in both male and female sticklebacks from Öresund between Skåne in southern Sweden and the Danish island of Zealand under seminatural conditions (Hellqvist et al., 2006). In males, *FSHβ* expression peaked in January and showed the lowest level in July. *LHβ* expression peaked in May and showed very low levels from June to September. At the highest peak of *LHβ* in May, kidney epithelium height and plasma 11KT levels were also at the highest levels. However, expression of both gonadotropic hormones was very low in the period when spermatogenesis commenced in June. The seasonal pattern of gonadotropic hormones may be induced by photoperiodic changes, because male sticklebacks have higher levels of mRNA for both *LHβ* and *FSHβ* under a long-day condition (16 h light:8 h dark) than those kept under a short-day condition (8 h light:16 h dark) (Shao

et al., 2013). The expression of gonadotropins changes not only seasonally but also depending on the nesting cycle of the stickleback. The expression of both $FSH\beta$ and $LH\beta$ is higher in nesting males than in post-breeding males (Hellqvist et al., 2001). In females, $LH\beta$ expression closely follows seasonal changes in ovarian mass; levels are low during winter and early spring, increase to a peak in late May, and decline to low levels again in June (Hellqvist et al., 2006).

Changes in feedback regulation between gonadotropins and sex steroids may play an important role in the control of seasonal reproduction in the three-spined stickleback (Shao et al., 2013). Under long-day conditions, $FSH\beta$ expression is reduced by castration but increased with androgen treatment, indicating positive feedback between androgens and FSH. In contrast, under short-day conditions, $FSH\beta$ expression is increased following castration and decreased with androgen treatment, indicating negative feedback between androgens and FSH under this condition. The negative feedback under short-day conditions may be a mechanism by which sexual maturation is inhibited in winter. Furthermore, the shift from negative to positive feedback with increasing photoperiods may be a mechanism by which full maturation occurs in spring. Thus, a shift in the feedback mechanisms between gonadotropins and sex steroids may function as a 'switch' for sexual maturation in the stickleback (Borg et al., 2004).

GnRH and kisspeptins
In vertebrates, GnRH generally stimulates the release of gonadotropic hormones from the anterior pituitary. Recently, kisspeptin, a neuropeptide encoded by a metastasis suppressor gene, *kiss*, and its receptor, G-coupled protein receptor 54 (GPR54), have been identified as important regulators of GnRH neurons and reproduction, especially at the beginning of puberty (Gopurappilly et al., 2013). Kisspeptins also play a role in the photoperiodic control of seasonal reproduction in several mammals such as the Siberian hamster (*Phodopus sungorus*) (Mason et al., 2007), while its role in seasonal reproduction in teleosts is less clear. Three-spined stickleback has two GnRH genes, *gnrh2* and *gnrh3*, but lacks *gnrh1* (O'Brien et al., 2012; Shao et al., 2015). In addition, although two paralogous kisspeptin genes (*kiss1* and *kiss2*) have been found in a number of teleost species, only *kiss2* has been found in the stickleback genome thus far (Felip et al., 2009; Tena-Sempere, 2006). A recent study reported gene expression patterns of *gnrh2*, *gnrh3*, *kiss2* and *gpr54* in the male three-spined stickleback over the course of sexual maturation induced by a shift from a short to a long photoperiod (Shao et al., 2019). When male sticklebacks were exposed to a long photoperiod, *gnrh3* expression was upregulated prior to the development of bright breeding colour, while the expression of *gnrh2*, *kiss2* and *gpr54* increased only after the onset of sexual maturation. The expression of $FSH\beta$ and $LH\beta$ increased simultaneously with *gnrh3* expression, implying that *gnrh3*, rather than *gnrh2*, *kiss2* and *gpr54*, may play an important role in the onset of sexual maturation. However, higher expression of *gnrh* genes under long photoperiod conditions were not observed in castrated males, suggesting the possibility that *gnrh* genes are upregulated by gonadal sex steroid hormones (Shao et al., 2015).

Melatonin
In vertebrates including sticklebacks, blood levels of melatonin are higher at night than during the day (Mayer et al., 1997b). In mammals, melatonin mediates photoperiodic effects on reproduction both in long-day breeders such as the Siberian

hamster, where it has an inhibitory effect (Carter and Goldman, 1983), and in short-day breeders such as sheep, where it has a stimulatory effect (Bittman et al., 1983). In contrast, there is little evidence that melatonin is involved in the control of seasonal reproduction in non-mammalian vertebrates (Mayer et al., 1997a). In the three-spined stickleback, melatonin injection has an inhibitory effect on reproduction (Borg and Ekström, 1981). However, this should be interpreted with caution because intraperitoneal injections could result in unnaturally high and transient peaks in plasma melatonin levels. In contrast, melatonin treatment via the ambient water failed to prevent the induction of maturation under a long photoperiod (Bornestaf et al., 2001; Mayer et al., 1997b). Seasonal changes in brain melatonin have been measured in three-spined sticklebacks from two Polish river populations exposed to annual environmental changes in their natural habitats (Sokołowska et al., 2004). In these two populations, brain melatonin levels were higher in spring and autumn than in winter and summer. Further studies are needed to confirm the physiological role of melatonin in seasonal reproduction in sticklebacks.

Thyroid stimulating hormone
In birds and mammals, thyroid stimulating hormone (TSH) induced by a long photoperiod is known to regulate seasonal reproduction (Nakane and Yoshimura, 2014; Shinomiya et al., 2014). TSH secreted from the pars tuberalis of the pituitary gland acts on TSH receptors (TSHRs) in the mediobasal hypothalamus to regulate type 2 iodothyronine deiodinase 2 (DIO2), an enzyme that converts prohormone thyroxine (T_4) to bioactive 3,5,3′-triiodothyronine (T_3). The local activation of thyroid hormone causes morphological changes in GnRH nerve terminals and regulates GnRH secretion (Yamamura et al., 2006). A recent study of masu salmon (*Oncorhynchus masou masou*) revealed that *TSH*, *TSHR* and *DIO2* are expressed in the saccus vasculosus (SV), an organ located at the bottom of the hypothalamus, posterior to the pituitary gland (Nakane et al., 2013). The isolated SV can respond to photoperiodic signals, and removal of the SV from fish prevents gonadal development induced by photoperiodic changes. These results suggest that the key elements for seasonal reproduction are conserved in fish, birds and mammals, while the organs or cells responsible for this response are diversified (Nakane and Yoshimura, 2014).

Our previous study suggested that different expression patterns of $TSH\beta2$ may be involved in the different seasonality between anadromous-marine and stream stickleback ecotypes (Kitano et al., 2010). In the three-spined stickleback, anadromous-marine ecotypes have higher plasma T_4 and T_3 levels than stream ecotypes. Although T_3 and T_4 do not show clear photoperiodic changes in either anadromous-marine or stream ecotypes, the expression of $TSH\beta2$ in the pituitary gland exhibits photoperiodic changes only in the anadromous-marine ecotypes. *Cis*-regulatory differences partially explain the differential expression of $TSH\beta2$ (Kitano et al., 2010). Importantly, there is a signature of divergent selection at the $TSH\beta2$ locus between anadromous-marine and stream ecotypes in North America (Kitano et al., 2010). Therefore, divergent natural selection on the reproductive strategy may act on the $TSH\beta2$ locus, resulting in differential expression patterns that are potentially involved in the regulation of seasonal reproduction in anadromous-marine and stream ecotypes. However, it is also possible that the different thyroid hormone levels are involved in differences in osmoregulation or metabolic rate between these two ecotypes (Kitano and Lema, 2013; Kitano et al., 2010).

Interestingly, in Atlantic herring (*Clupea hearengus*), the TSHR locus shows the most convincing association with spawning timing between spring- and autumn-spawning populations (Lamichhaney et al., 2017). Furthermore, the TSHR locus has the most striking selective sweep in the domestic chicken (*Gallus gallus domesticus*), which lost the strict regulation of seasonal reproduction during domestication (Rubin et al., 2010). These studies may imply convergent evolution of the TSH pathway underlying variation in reproductive seasonality in diverse taxa.

The future of physiological genomics of seasonal reproduction in sticklebacks

The diversity in the breeding season of the three-spined stickleback demonstrates that this species is an excellent biological resource for further investigation of the genetic basis underlying the evolution of life history traits.

Rapid advances in next-generation sequencing will enable us to identify genomic loci responsible for variation in seasonal reproduction. First, a genome-wide association study (GWAS) would be useful to identify single-nucleotide polymorphisms (SNPs) associated with variation in seasonal reproduction. The GWAS approach has been used to successfully identify SNPs associated with age at maturity in salmon (Ayllon et al., 2015; Gutierrez et al., 2015; Mohamed et al., 2019) and flowering time in many plant species (Grabowski et al., 2017; Romero Navarro et al., 2017; Urrestarazu et al., 2017; Zhang et al., 2015). Second, a genome-wide scan for signatures of selection would also help to identify candidate genes. In the three-spined stickleback, several genes responsible for repeated morphological evolution in freshwater ecotypes, such as the *ectodysplasin A* gene for armour plate reduction and the *paired-like homeodomain 1* gene for pelvic reduction, show signatures of natural selection (Chan et al., 2010; Colosimo et al., 2005; Miller et al., 2007). Therefore, a search for genomic signatures of divergent selection between ecotypes or between stream ecotypes from different latitudes would be promising for identifying candidate genes or mutations underlying variation in seasonal reproduction. Third, quantitative trait loci (QTL) mapping has been used to identified genes and mutations responsible for divergent morphological and behavioural phenotypes between ecotypes (Chan et al., 2010; Colosimo et al., 2005; Glazer et al., 2014; Greenwood et al., 2013; Miller et al., 2007; Shapiro et al., 2004). However, few physiological traits related to life history have been mapped to date using QTL analysis in sticklebacks (Ishikawa et al., 2019). Finally, transcriptome analysis would also be useful to search for genes involved in seasonal reproduction, given the fact that some genes, such as *gnrh3* and *TSHβ2*, demonstrate substantial changes in expression following changes in photoperiod. A combination of QTL and transcriptomics, expression QTL (eQTL) analysis (i.e. QTL analysis of transcript levels), will also improve our understanding of the genetic architecture and regulatory cascades responsible for the evolution of different life history strategies in multiple environmental conditions (Ishikawa et al., 2017).

Although GWAS, QTL mapping and other -omics approaches can provide us with a list of candidate genes or mutations, gene manipulation is necessary to confirm causal association with phenotype. In the three-spined stickleback, genome editing and transgenic techniques have already been established (Chan et al., 2010; Colosimo et al., 2005; Erickson et al., 2016; Hart and Miller, 2017; Hosemann et al., 2004; Howes et al., 2017; Ishikawa et al., 2019; Wucherpfennig et al., 2019). These tools allow us to overexpress and knock out genes to directly test the function of candidate genes *in vivo*. Genetic manipulations also enable us to investigate the pleiotropic effects of candidate genes. Importantly, most of the candidate hormones or hormone-related genes involved in seasonal reproduction in sticklebacks can affect a variety of target tissues to regulate the expression of multiple phenotypic traits. Generally, such pleiotropic effects of hormonal pathways may act as a constraint on independent phenotypic evolution (Ketterson and Nolan, 1999; Kitano et al., 2014). However, when changes in a suite of traits controlled by the same hormones are favourable, genetic changes in the hormonal pathway may simultaneously change multiple traits at one time and enable animals to rapidly adapt to new environments. The transition from the ancestral marine environment to freshwater generally accompanies evolutionary changes in several life history traits such as an increase in egg size, a reduction in clutch size, a decline in growth rate and possibly greater variation in average maximum size (Baker, 1994). Using genetically engineered fish, we will be able to investigate the pleiotropic effects of candidate genes. Finally, gene replacement has been successfully conducted in several fish species (Kimura et al., 2014; Watakabe et al., 2018), which may be possible in sticklebacks in the near future.

In conclusion, the presence of ecotypes with diverse reproductive seasonality and the availability of genetic and genomic tools will make the three-spined stickleback a good model for studying the genetic basis of the evolution of life history traits. Using the stickleback as a model, we will be able to improve our understanding of the genetic factors underlying variation in the physiological signalling pathways involved in the integration of photoperiodic signals to regulate reproduction.

References
Aguirre, W. E., Ellis, K. E., Kusenda, M. and Bell, M. A. (2008). Phenotypic variation and sexual dimorphism in anadromous threespine stickleback: implications for postglacial adaptive radiation. *Biol. J. Linn. Soc.* **95**, 465-478. doi:10.1111/j.1095-8312.2008.01075.x

Allen, J. R. M. and Wootton, R. J. (1984). Temporal patterns in diet and rate of food consumption of the three-spined stickleback (Gasterosteus aculeatus L.) in Llyn Frongoch, an upland Welsh lake. *Freshw. Biol.* **14**, 335-346. doi:10.1111/j.1365-2427.1984.tb00158.x

Andersson, E., Borg, B. and Goos, H. J. T. (1992). Temperature, but not photoperiod, influences gonadotropin-releasing hormone binding in the pituitary of the three-spined stickleback, gasterosteus aculeatus. *Gen. Comp. Endocrinol.* **88**, 111-116. doi:10.1016/0016-6480(92)90199-T

Ayllon, F., Kjærner-Semb, E., Furmanek, T., Wennevik, V., Solberg, M. F., Dahle, G., Taranger, G. L., Glover, K. A., Almén, M. S., Rubin, C. J. et al. (2015). The vgll3 locus controls age at maturity in wild and domesticated Atlantic Salmon (Salmo salar L.) males. *PLoS Genet.* **11**, e1005628. doi:10.1371/journal.pgen.1005628

Baggerman, B. (1957). An experimental study on the timing of breeding and migration in the three-spipned stickleback (*Gasterosteus aculeatus* L.). *Arch. Neerl. Zool.* **12**, 105. doi:10.1163/036551658X00010

Baggerman, B. (1972). Photoperiodic responses in the stickleback and their control by a daily rythm of photosensitivity. *Gen. Comp. Endocrinol.* **3**, 466-476. doi:10.1016/0016-6480(72)90177-3

Baggerman, B. (1984). The role of biological rhythms in the photoperiodic regulation of seasonal breeding in the stickleback *Gasterosteus a culea tus* L. *Neth. J. Zool.* **35**, 14-31. doi:10.1163/002829685X00046

Baker, J. R. (1939). The relation between latitude and breeding seasons in birds. *Proc. Zool. Soc. Lond.* **A108**, 557-582. doi:10.1111/j.1096-3642.1939.tb00042.x

Baker, J. A. (1994). Life history variation in female threespine stickleback. In *The Evolutionary Biology of the Threespine Stickleback* (ed. M. A. Bell and S. A. Foster), pp. 144-187. Oxford University Press.

Bakker, T. C. M. and Mundwiler, B. (1994). Female mate choice and male red coloration in a natural three-spined stickleback (Gasterosteus aculeatus) population. *Behav. Ecol.* **5**, 74-80. doi:10.1093/beheco/5.1.74

Bakker, T. C. M. and Mundwiler, B. (1999). Pectoral fin size in a fish species with paternal care: a condition-dependent sexual trait revealing infection status. *Freshw. Biol.* **41**, 543-551. doi:10.1046/j.1365-2427.1999.00403.x

Baskin, J. N. (1974). *Survey of unarmored threespine stickleback (Gasterosteus aculeates williamsoni) in the upper Santa Clara River drainage*. Final Report for the Bureau of Sport Fisheries and Wildlife, Contract No. 14-16-0001-5387SE.

Bell, M. A. and Foster, S. A. (1994). *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press.

Bertin, L. (1925). Recherches bionomiques, biometriques et systematiques sur les Epinoches (Gasterosteides). *Ann. Inst. Oceanogr. Monaco* **2**, 1-204.

Bittman, E. L., Dempsey, R. J. and Karsch, F. J. (1983). Pineal melatonin secretion drives the reproductive response to daylength in the ewe. *Endocrinology* **113**, 2276-2283. doi:10.1210/endo-113-6-2276

Bolnick, D. I., Shim, K. C., Schmerer, M. and Brock, C. D. (2015). Population-specific covariation between immune function and color of nesting male threespine stickleback. *PLoS ONE* **10**, e0126000. doi:10.1371/journal.pone.0126000

Borg, B. (1981). Effects of methyltestosterone on spermatogenesis and secondary sexual characters in the three-spined stickleback (Gasterosteus aculeatus L.). *Gen. Comp. Endocrinol.* **44**, 177-180. doi:10.1016/0016-6480(81)90245-8

Borg, B. (1982a). Extraretinal photoreception involved in photoperiodic effects on reproduction in male three-spined sticklebacks, *Gasterosteus aculeatus*. *Gen. Comp. Endocrinol.* **47**, 84-87. doi:10.1016/0016-6480(82)90087-9

Borg, B. (1982b). Seasonal effects of photoperiod and temperature on spermatogenesis and male secondary sexual characters in the three-spined stickleback, Gasterosteus aculeatus L. *Can. J. Zool.* **60**, 3377-3386. doi:10.1139/z82-427

Borg, B. (1994). Androgens in teleost fishes. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* **109**, 219-245. doi:10.1016/0742-8413(94)00063-G

Borg, B. (2010). Photoperiodism in fishes. In *Photoperiodism: the Biological Calendar* (ed. R. J. Nelson, D. L. Denlinger and D. E. Somers), pp. 371-398. Oxford: Oxford University Press.

Borg, B. and Ekström, P. (1981). Gonadal effects of melatonin in the three-spined stickleback, Gasterosteus aculeatus L., during different seasons and photoperiods. *Reprod. Nutr. Dev.* **21**, 919-927. doi:10.1051/rnd:19810704

Borg, B. and Veen, T. V. (1982). Seasonal effects of photoperiod and temperature on the ovary of the three-spined stickleback, Gasterosteus aculeatus L. *Can. J. Zool.* **60**, 3387-3393. doi:10.1139/z82-428

Borg, B., Antonopoulou, E., Andersson, E., Carlberg, T. and Mayer, I. (1993). Effectiveness of several androgens in stimulating kidney hypertrophy, a secondary sexual character, in castrated male three-spined sticklebacks, Gasterosteus aculeatus. *Can. J. Zool.* **71**, 2327-2329. doi:10.1139/z93-326

Borg, B., Bornestaf, C., Hellqvist, A., Schmitz, M. and Mayer, I. (2004). Mechanisms in the photoperiodic control of reproduction in the stickleback. *Behaviour* **141**, 1521-1530. doi:10.1163/1568539042948213

Bornestaf, C. and Borg, B. (2000). Endogenous breeding cycles in male threespine sticklebacks, *Gasterosteus aculeatus*. *Behaviour* **137**, 921-932. doi:10.1163/156853900502538

Bornestaf, C., Mayer, I. and Borg, B. (2001). Melatonin and maturation pace in female three-spined stickleback, *Gasterosteus aculeatus*. *Gen. Comp. Endocrinol.* **122**, 341-348. doi:10.1006/gcen.2001.7640

Bradshaw, W. E. and Holzapfel, C. M. (2007). Evolution of animal photoperiodism. *Ann. Rev. Ecol. Evol. Syst.* **38**, 1-25. doi:10.1146/annurev.ecolsys.37.091305.110115

Bronson, F. H. (1985). Mammalian reproduction: an ecological perspective. *Biol. Reprod.* **32**, 1-26. doi:10.1095/biolreprod32.1.1

Burton, D. (1979). Sexual dimorphism in the integumentary tissues of threespine stickleback, *Gasterosteus aculeatus* form leiurus. *Copeia* **1979**, 533-535. doi:10.2307/1443236

Caldecutt, W. J., Bell, M. A. and Buckland-Nicks, J. A. (2001). Sexual dimorphism and geographic variation in dentition of threespine stickleback, Gasterosteus aculeatus. *Copeia* **2001**, 936-944. doi:10.1643/0045-8511(2001)001[0936:SDAGVI]2.0.CO;2

Carter, D. S. and Goldman, B. D. (1983). Antigonadal effects of timed melatonin infusion in pinealectomized male Djungarian hamsters (Phodopus sungorus sungorus): duration is the critical parameter. *Endocrinology* **113**, 1261-1267. doi:10.1210/endo-113-4-1261

Chan, Y. F., Marks, M. E., Jones, F. C., Villarreal, G., Shapiro, M. D., Brady, S. D., Southwick, A. M., Absher, D. M., Grimwood, J., Schmutz, J. et al. (2010). Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a pitx1 enhancer. *Science* **327**, 302-305. doi:10.1126/science.1182213

Colosimo, P. F., Hosemann, K. E., Balabhadra, S., Villarreal, G., Dickson, M., Grimwood, J., Schmutz, J., Myers, R. M., Schluter, D. and Kingsley, D. M. (2005). Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. *Science* **307**, 1928-1933. doi:10.1126/science.1107239

Coyne, J. A. and Orr, H. A. (2004). *Speciation*: Sinauer.

Craig-Bennett, A. (1931). The reproductive cycle of the three-spined stickleback, *Gasterosteus aculeatus*, Linn. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **219**, 197-279. doi:10.1098/rstb.1931.0005

Crivelli, A. J. and Britton, R. H. (1987). Life history adaptations of Gasterosteus aculeatus in a Mediterranean wetland. *Environ. Biol. Fishes* **18**, 109-125. doi:10.1007/BF00002599

Dauod, H. A., Bolger, T. and Bracken, J. J. (1985). Studies on the three-spined stickleback Gasterosteus aculeatus L. from an upland Irish reservoir system. *Irish Fish. Invest. Series A No. 27* **17**.

Engel, L. J. (1971). *Evaluation of Sport Fish Stocking on the Kenai Peninsula-Cook Inlet area, July 1, 1970 to June 30, 1971. Project F-9-3, Job g-11-F*, Vol. **12**, pp. 1-34. Alaska Department of Fish and Game.

Erickson, P. A., Ellis, N. A. and Miller, C. T. (2016). Microinjection for transgenesis and genome editing in threespine sticklebacks. *JoVE* e54055. doi:10.3791/54055

Fang, B., Merilä, J., Ribeiro, F., Alexandre, C. M. and Momigliano, P. (2018). Worldwide phylogeny of three-spined sticklebacks. *Mol. Phylogenet. Evol.* **127**, 613-625. doi:10.1016/j.ympev.2018.06.008

Felip, A., Zanuy, S., Pineda, R., Pinilla, L., Carrillo, M., Tena-Sempere, M. and Gómez, A. (2009). Evidence for two distinct KiSS genes in non-placental vertebrates that encode kisspeptins with different gonadotropin-releasing activities in fish and mammals. *Mol. Cell. Endocrinol.* **312**, 61-71. doi:10.1016/j.mce.2008.11.017

Glazer, A. M., Cleves, P. A., Erickson, P. A., Lam, A. Y. and Miller, C. T. (2014). Parallel developmental genetic features underlie stickleback gill raker evolution. *EvoDevo* **5**, 19. doi:10.1186/2041-9139-5-19

Golovin, P. V., Bakhvalova, A. E., Ivanov, M. V., Ivanova, T. S., Smirnova, K. A. and Lajus, D. L. (2019). Sex-biased mortality of marine threespine stickleback *Gasterosteus aculeatus* L. during their spawning period in the White Sea. *Evol. Ecol. Res.* **20**, 279-285.

Gopurappilly, R., Ogawa, S. and Parhar, I. S. (2013). Functional significance of GnRH and kisspeptin, and their cognate receptors in teleost reproduction. *Front. Endocrinol.* **4**, 24-24. doi:10.3389/fendo.2013.00024

Grabowski, P. P., Evans, J., Daum, C., Deshpande, S., Barry, K. W., Kennedy, M., Ramstein, G., Kaeppler, S. M., Buell, C. R., Jiang, Y. et al. (2017). Genome-wide associations with flowering time in switchgrass using exome-capture sequencing data. *New Phytol.* **213**, 154-169. doi:10.1111/nph.14101

Graham, M. A., Earley, R. L., Baker, J. A. and Foster, S. A. (2018). Evolution of steroid hormones in reproductive females of the threespine stickleback fish. *Gen. Comp. Endocrinol.* **268**, 71-79. doi:10.1016/j.ygcen.2018.08.003

Greenwood, A. K., Wark, A. R., Yoshida, K. and Peichel, C. L. (2013). Genetic and neural modularity underlie the evolution of schooling behavior in threespine sticklebacks. *Curr. Biol.* **23**, 1884-1888. doi:10.1016/j.cub.2013.07.058

Gutierrez, A. P., Yanez, J. M., Fukui, S., Swift, B. and Davidson, W. S. (2015). Genome-wide association study (GWAS) for growth rate and age at sexual maturation in Atlantic salmon (Salmo salar). *PLoS ONE* **10**, e0119730. doi:10.1371/journal.pone.0119730

Hagen, D. W. (1967). Isolating mechanisms in threespine sticklebacks (*Gasterosteus*). *J. Fish. Res. Broad Can.* **24**, 1637-1692. doi:10.1139/f67-138

Hart, J. C. and Miller, C. T. (2017). Sequence-based mapping and genome editing reveal mutations in stickleback *Hps5* cause oculocutaneous slbinism and the *casper* phenotype. *G3 (Bethesda)* **7**, 3123. doi:10.1534/g3.117.1125

Hellqvist, A., Schmitz, M., Lindberg, C., Olsson, P.-E. and Borg, B. (2001). LH-β and FSH-β mRNA expression in nesting and post-breeding three-spined stickleback, Gasterosteus aculeatus, and effects of castration on expression of LH-β, FSH-β and spiggin mRNA. *Fish Physiol. Biochem.* **25**, 311-317. doi:10.1023/A:1023210407163

Hellqvist, A., Schmitz, M., Mayer, I. and Borg, B. (2006). Seasonal changes in expression of LH-β and FSH-β in male and female three-spined stickleback, Gasterosteus aculeatus. *Gen. Comp. Endocrinol.* **145**, 263-269. doi:10.1016/j.ygcen.2005.09.012

Hoar, W. S. (1962). Hormones and the reproductive behaviour of the male three-spined stickleback (Gasterosteus aculeatus). *Anim. Behav.* **10**, 247-266. doi:10.1016/0003-3472(62)90049-0

Hoffmann, E. and Borg, B. (2006). Sex differences in pectoral muscles but not in pectoral fins in the three-spined stickleback Gasterosteus aculeatus. *J. Fish Biol.* **68**, 1451-1459. doi:10.1111/j.0022-1112.2006.001030.x

Hosemann, K. E., Colosimo, P. F., Summers, B. R. and David, M. (2004). A simple and efficient microinjection protocol for making transgenic sticklebacks. *Behaviour* **141**, 1345-1355. doi:10.1163/1568539042948097

Howes, T. R., Summers, B. R. and Kingsley, D. M. (2017). Dorsal spine evolution in threespine sticklebacks via a splicing change in MSX2A. *BMC Biol.* **15**, 115. doi:10.1186/s12915-017-0456-5

Indjeian, V. B., Kingman, G. A., Jones, F. C., Schmutz, J., Myers, R. M., Kingsley, D. M., Indjeian, V. B., Kingman, G. A., Jones, F. C., Guenther, C. A. et al. (2016). Evolving new skeletal traits by *cis*-regulatory changes in bone morphogenetic proteins. *Cell* **164**, 45-56. doi:10.1016/j.cell.2015.12.007

Ishikawa, A. and Kitano, J. (2019). Data from: Diversity in reproductive seasonality in the threespine stickleback, Gasterosteus aculeatus. *Dryad Digital Repository*. https://doi.org/10.5061/dryad.pb7v936

Ishikawa, A., Kusakabe, M., Yoshida, K., Ravinet, M., Makino, T., Toyoda, A., Fujiyama, A. and Kitano, J. (2017). Different contributions of local- and distant-regulatory changes to transcriptome divergence between stickleback ecotypes. *Evolution* **71**, 565-581. doi:10.1111/evo.13175

Ishikawa, A., Kabeya, N., Ikeya, K., Kakioka, R., Cech, J. N., Osada, N., Leal, M. C., Inoue, J., Kume, M., Toyoda, A. et al. (2019). A key metabolic gene for recurrent freshwater colonization and radiation in fishes. *Science* **364**, 886. doi:10.1126/science.aau5656

Ivanova, T. S., Ivanov, M. V., Bakhvalova, A. E., Polyakova, N. V., Golovin, P. V., Kucheryavyy, A. V., Yurtseva, A. O., Smirnova, K. A. and Lajus, D. L. (2019). Homing ability and site fidelity of marine threespine stickleback on spawning grounds. *Evol. Ecol. Res.* **20**, 297-315.

Jones, F. C., Brown, C., Pemberton, J. M. and Braithwaite, V. A. (2006). Reproductive isolation in a threespine stickleback hybrid zone. *J. Evol. Biol.* **19**, 1531-1544. doi:10.1111/j.1420-9101.2006.01122.x

Jones, F. C., Chan, Y. F., Schmutz, J., Grimwood, J., Brady, S. D., Southwick, A. M., Absher, D. M., Myers, R. M., Reimchen, T. E., Deagle, B. E. et al. (2012). A genome-wide SNP genotyping array reveals patterns of global and repeated species-pair divergence in sticklebacks. *Curr. Biol.* **22**, 83-90. doi:10.1016/j.cub.2011.11.045

Karve, A. D., Von Hippel, F. A. and Bell, M. A. (2008). Isolation between sympatric anadromous and resident threespine stickleback species in Mud Lake, Alaska. *Environ. Biol. Fishes* **81**, 287-296. doi:10.1007/s10641-007-9200-2

Ketterson, E. D. and Nolan, J. V. (1999). Adaptation, exaptation, and constraint: a hormonal perspective. *Am. Nat.* **154**, S4-S25. doi:10.1086/303280

Kimura, Y., Hisano, Y., Kawahara, A. and Higashijima, S.-l. (2014). Efficient generation of knock-in transgenic zebrafish carrying reporter/driver genes by CRISPR/Cas9-mediated genome engineering. *Sci. Rep.* **4**, 6545. doi:10.1038/srep06545

Kitano, J. and Lema, S. C. (2013). Divergence in thyroid hormone concentrations between juveniles of marine and stream ecotypes of the threespine stickleback (Gasterosteus aculeatus). *Evol. Ecol. Res.* **15**, 143-153.

Kitano, J., Mori, S. and Peichel, C. L. (2007). Sexual dimorphism in the external morphology of the threespine stickleback (Gasterosteus aculeatus). *Copeia* **2007**, 336-349. doi:10.1643/0045-8511(2007)7[336:SDITEM]2.0.CO;2

Kitano, J., Lema, S. C., Luckenbach, J. A., Mori, S., Kawagishi, Y., Kusakabe, M., Swanson, P. and Peichel, C. L. (2010). Adaptive divergence in the thyroid hormone signaling pathway in the stickleback radiation. *Curr. Biol.* **20**, 2124-2130. doi:10.1016/j.cub.2010.10.050

Kitano, J., Kawagishi, Y., Mori, S., Peichel, C. L., Makino, T., Kawata, M. and Kusakabe, M. (2011). Divergence in sex steroid hormone signaling between sympatric species of Japanese threespine stickleback. *PLoS ONE* **6**, e29253. doi:10.1371/journal.pone.0029253

Kitano, J., Ishikawa, A., Kume, M. and Mori, S. (2012). Physiological and genetic basis for variation in migratory behavior in the three-spined stickleback, *Gasterosteus aculeatus*. *Ichthyol. Res.* **59**, 293-303. doi:10.1007/s10228-012-0289-8

Kitano, J., Ishikawa, A. and Lema, S. (2014). Integrated genomics approaches in evolutionary and ecological endocrinology. In *Ecological Genomics*, Vol. 781 (ed. C. R. Landry and N. Aubin-Horth), pp. 299-319: Springer Netherlands.

Kotrschal, A., Räsänen, K., Kristjánsson, B. K., Senn, M. and Kolm, N. (2012). Extreme sexual brain size dimorphism in sticklebacks: a consequence of the cognitive challenges of sex and parenting? *PLoS ONE* **7**, e30055. doi:10.1371/journal.pone.0030055

Kume, M., Kitamura, T., Takahashi, H. and Goto, A. (2005). Distinct spawning migration patterns in sympatric Japan Sea and Pacific Ocean forms of threespine stickleback Gasterosteus aculeatus. *Ichthyol. Res.* **52**, 189-193. doi:10.1007/s10228-005-0269-3

Lachance, S., Magnan, P. and FitzGerald, G. J. (1987). Temperature preferences of three sympatric sticklebacks (Gasterosteidae). *Can. J. Zool.* **65**, 1573-1576. doi:10.1139/z87-245

Lamichhaney, S., Fuentes-Pardo, A. P., Rafati, N., Ryman, N., McCracken, G. R., Bourne, C., Singh, R., Ruzzante, D. E. and Andersson, L. (2017). Parallel adaptive evolution of geographically distant herring populations on both sides of the North Atlantic Ocean. *Proc. Natl Acad. Sci. USA* **114**, E3452-E3461. doi:10.1073/pnas.1617728114

Leinonen, T., Cano, J. M., Mäkinen, H. and Merilä, J. (2006). Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *J. Evol. Biol.* **19**, 1803-1812. doi:10.1111/j.1420-9101.2006.01182.x

Leinonen, T., Cano, J. M. and Merilä, J. (2011). Genetic basis of sexual dimorphism in the threespine stickleback Gasterosteus aculeatus. *Heredity* **106**, 218-227. doi:10.1038/hdy.2010.104

Lenth, R. V. (2016). Least-squares means: the r package lsmeans. *J. Stat. Softw.* **69**. doi:10.18637/jss.v069.i01

Marques, D. A., Taylor, J. S., Jones, F. C., Di Palma, F., Kingsley, D. M. and Reimchen, T. E. (2017). Convergent evolution of SWS2 opsin facilitates adaptive radiation of threespine stickleback into different light environments. *PLoS Biol.* **15**, e2001627. doi:10.1371/journal.pbio.2001627

Mason, A. O., Greives, T. J., Scotti, M.-A. L., Levine, J., Frommeyer, S., Ketterson, E. D., Demas, G. E. and Kriegsfeld, L. J. (2007). Suppression of kisspeptin expression and gonadotropic axis sensitivity following exposure to inhibitory day lengths in female Siberian hamsters. *Horm. Behav.* **52**, 492-498. doi:10.1016/j.yhbeh.2007.07.004

Mayer, I., Borg, B. and Schulz, R. (1990). Seasonal changes in and effect of castration/androgen replacement on the plasma levels of five androgens in the male three-spined stickleback, Gasterosteus aculeatus L. *Gen. Comp. Endocrinol.* **79**, 23-30. doi:10.1016/0016-6480(90)90084-Y

Mayer, I., Bornestaf, C. and Borg, B. (1997a). Melatonin in non-mammalian vertebrates: Physiological role in reproduction? *Comp. Biochem. Physiol. A Physiol.* **118**, 515-531. doi:10.1016/S0300-9629(96)00468-9

Mayer, I., Bornestaf, C., Wetterberg, L. and Borg, B. (1997b). Melatonin does not prevent long photoperiod stimulation of secondary sexual characters in the male three-spined stickleback Gasterosteus aculeatus. *Gen. Comp. Endocrinol.* **108**, 386-394. doi:10.1006/gcen.1997.6985

Mayr, E. (1963). *Animal Species and Evolution*: Belknap Press of Harvard University Press.

McGee, M. D. and Wainwright, P. C. (2013). Sexual dimorphism in the feeding mechanism of threespine stickleback. *J. Exp. Biol.* **216**, 835-840. doi:10.1242/jeb.074948

McPhail, J. D. (1969). Predation and the evolution of a stickleback (*Gasterosteus*). *J. Fish. Res. Board Can.* **26**, 3183-3208. doi:10.1139/f69-301

Milinski, M. and Bakker, T. C. M. (1990). Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344**, 330-333. doi:10.1038/344330a0

Miller, C. T., Beleza, S., Pollen, A. A., Schluter, D., Kittles, R. A., Shriver, M. D. and Kingsley, D. M. (2007). *Cis* regulatory changes in *Kit ligand* expression and parallel evolution of pigmentation in sticklebacks and humans. *Cell* **131**, 1179-1189. doi:10.1016/j.cell.2007.10.055

Mohamed, A. R., Verbyla, K. L., Al-Mamun, H. A., McWilliam, S., Evans, B., King, H., Kube, P. and Kijas, J. W. (2019). Polygenic and sex specific architecture for two maturation traits in farmed Atlantic salmon. *BMC Genomics* **20**, 139. doi:10.1186/s12864-019-5525-4

Mori, S. (1985). Reproductive behaviour of the landlocked three-spined stickleback, Gasterosteus aculeatus microcephalus, in Japan: I. The year-long prolongation of the breeding period in waterbodies with springs. *Behaviour* **93**, 20-35. doi:10.1163/156853986X00702

Nakane, Y. and Yoshimura, T. (2014). Universality and diversity in the signal transduction pathway that regulates seasonal reproduction in vertebrates. *Front. Neurosci.* **8**, 1-7. doi:10.3389/fnins.2014.00115

Nakane, Y., Ikegami, K., Iigo, M., Ono, H., Takeda, K., Takahashi, D., Uesaka, M., Kimijima, M., Hashimoto, R., Arai, N. et al. (2013). The saccus vasculosus of fish is a sensor of seasonal changes in day length. *Nat. Commun.* **4**, 317. doi:10.1038/ncomms3108

Nosil, P. (2012). *Ecological Speciation*: Oxford University Press.

O'Brien, C. S., Bourdo, R., Bradshaw, W. E., Holzapfel, C. M. and Cresko, W. A. (2012). Conservation of the photoperiodic neuroendocrine axis among vertebrates: evidence from the teleost fish, Gasterosteus aculeatus. *Gen. Comp. Endocrinol.* **178**, 19-27. doi:10.1016/j.ygcen.2012.03.010

O'Brown, N. M., Summers, B. R., Jones, F. C., Brady, S. D. and Kingsley, D. M. (2015). A recurrent regulatory change underlying altered expression and Wnt response of the stickleback armor plates gene EDA. *eLife* **4**, e05290. doi:10.7554/eLife.05290

Ravinet, M., Yoshida, K., Shigenobu, S., Toyoda, A., Fujiyama, A. and Kitano, J. (2018). The genomic landscape at a late stage of stickleback speciation: high genomic divergence interspersed by small localized regions of introgression. *PLoS Genet.* **14**, e1007358. doi:10.1371/journal.pgen.1007358

Reimchen, T. E. (1989). Loss of nuptial color in threespine sticklebacks (Gasterosteus aculeatus). *Evolution* **43**, 450-460. doi:10.1111/j.1558-5646.1989.tb04239.x

Roff, D. A. (2002). *Life History Evolution*. Sunderland, MA: Sinauer.

Romero Navarro, J. A., Willcox, M., Burgueño, J., Romay, C., Swarts, K., Trachsel, S., Preciado, E., Terron, A., Delgado, H. V., Vidal, V. et al. (2017). A study of allelic diversity underlying flowering-time adaptation in maize landraces. *Nat. Genet.* **49**, 476. doi:10.1038/ng.3784

Rubin, C.-J., Zody, M. C., Eriksson, J., Meadows, J. R. S., Sherwood, E., Webster, M. T., Jiang, L., Ingman, M., Sharpe, T., Ka, S. et al. (2010). Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature* **464**, 587-591. doi:10.1038/nature08832

Samuk, K., Iritani, D. and Schluter, D. (2014). Reversed brain size sexual dimorphism accompanies loss of parental care in white sticklebacks. *Ecol. Evol.* **4**, 3236-3243. doi:10.1002/ece3.1175

Shao, Y. T., Arvidsson, M., Trombley, S., Schulz, R. W., Schmitz, M. and Borg, B. (2013). Androgen feedback effects on LH and FSH, and photoperiodic control of reproduction in male three-spined sticklebacks, Gasterosteus aculeatus. *Gen. Comp. Endocrinol.* **182**, 16-23. doi:10.1016/j.ygcen.2012.10.017

Shao, Y. T., Tseng, Y. C., Chang, C.-H., Yan, H. Y., Hwang, P. P. and Borg, B. (2015). GnRH mRNA levels in male three-spined sticklebacks, Gasterosteus aculeatus, under different reproductive conditions. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **180**, 6-17. doi:10.1016/j.cbpa.2014.10.008

Shao, Y. T., Roufidou, C., Chung, P. C. and Borg, B. (2019). Changes in kisspeptin, GnRH, and gonadotropin mRNA levels in male Threespine stickleback (*Gasterosteus aculeatus*) during photoperiod-induced sexual maturation. *Evol. Ecol. Res.* **20**, 317-329.

Shapiro, M. D., Marks, M. E., Peichel, C. L., Blackman, B. K., Nereng, K. S., Jónsson, B., Schluter, D. and Kingsley, D. M. (2004). Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* **428**, 717. doi:10.1038/nature02415

Shinomiya, A., Shimmura, T., Nishiwaki-Ohkawa, T. and Yoshimura, T. (2014). Regulation of seasonal reproduction by hypothalamic activation of thyroid hormone. *Front. Endocrinol.* **5**, 12. doi:10.3389/fendo.2014.00012

Sokołowska, E. and Kulczykowska, E. (2006). Annual reproductive cycle in two free living populations of three-spined stickleback (*Gasterosteus aculeatus* L.): patterns of ovarian and testicular development. *Oceanologia* **48**, 103-124.

Sokołowska, E. and Kulczykowska, E. (2009). Environmental influence on maturation and dominance relationships in the three-spined stickleback (Gasterosteus aculeatus L.): temperature competes with photoperiod for primacy. *Oceanol. Hydrobiol. St.* **38**, 161. doi:10.2478/v10009-009-0042-4

Sokołowska, E., Kalamarz, H. and Kulczykowska, E. (2004). Seasonal changes in brain melatonin concentration in the three-spined stickleback (Gasterosteus aculeatus): towards an endocrine calendar. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **139**, 365-369. doi:10.1016/j.cbpb.2004.10.001

Spoljaric, M. A. and Reimchen, T. E. (2008). Habitat-dependent reduction of sexual dimorphism in geometric body shape of Haida Gwaii threespine stickleback. *Biol. J. Linn. Soc.* **95**, 505-516. doi:10.1111/j.1095-8312.2008.01068.x

Stearns, S. C. (1992). *The Evolution of Life Histories*: OUP Oxford.

Tena-Sempere, M. (2006). GPR54 and kisspeptin in reproduction. *Hum. Reprod. Update* **12**, 631-639. doi:10.1093/humupd/dml023

Tinbergen, N. (1951). *The Study of Instinct*: Clarendon Press.

Urrestarazu, J., Muranty, H., Denancé, C., Leforestier, D., Ravon, E., Guyader, A., Guisnel, R., Feugey, L., Aubourg, S., Celton, J.-M. et al. (2017). Genome-wide association mapping of flowering and ripening periods in apple. *Front. Plant Sci.* **8**, 1923-1923. doi:10.3389/fpls.2017.01923

Wai, E. H. and Hoar, W. S. (1963). The secondary sex characters and reproductive behavior of gonadectomized sticklebacks treated with methyl testosterone. *Can. J. Zool.* **41**, 611-628. doi:10.1139/z63-034

Watakabe, I., Hashimoto, H., Kimura, Y., Yokoi, S., Naruse, K. and Higashijima, S.-I. (2018). Highly efficient generation of knock-in transgenic medaka by CRISPR/Cas9-mediated genome engineering. *Zool. Lett.* **4**, 3. doi:10.1186/s40851-017-0086-3

Wedekind, C., Meyer, P., Frischknecht, M., Niggli, U. A. and Pfander, H. (1998). Different carotenoids and potential information content of red coloration of male three-spined stickleback. *J. Chem. Ecol.* **24**, 787-801. doi:10.1023/A:1022365315836

Wootton, R. (1976). *The Biology of the Sticklebacks*. London: Academic Press.

Wootton, R. (1984). *A Functional Biology of Sticklebacks*. California: University of California Press.

Wootton, R. J., Evans, G. W. and Mills, L. (1978). Annual cycle in female Three-spined sticklebacks (Gasterosteus aculeatus L.) from an upland and lowland population. *J. Fish Biol.* **12**, 331-343. doi:10.1111/j.1095-8649.1978.tb04178.x

Wucherpfennig, J. I., Miller, C. T. and Kingsley, D. M. (2019). Efficient CRISPR-Cas9 editing of major evolutionary loci in sticklebacks. *Evol. Ecol. Res.* **20**.

Xie, K. T., Wang, G., Thompson, A. C., Wucherpfennig, J. I., Reimchen, T. E., MacColl, A. D. C., Schluter, D., Bell, M. A., Vasquez, K. M. and Kingsley, D. M. (2019). DNA fragility in the parallel evolution of pelvic reduction in stickleback fish. *Science* **363**, 81. doi:10.1126/science.aan1425

Yamamura, T., Yasuo, S., Hirunagi, K., Ebihara, S. and Yoshimura, T. (2006). T3 implantation mimics photoperiodically reduced encasement of nerve terminals by glial processes in the median eminence of Japanese quail. *Cell Tissue Res.* **324**, 175-179. doi:10.1007/s00441-005-0126-8

Yeates-Burghart, Q. S., O'Brien, C., Cresko, W. A., Holzapfel, C. M. and Bradshaw, W. E. (2009). Latitudinal variation in photoperiodic response of the three-spined stickleback Gasterosteus aculeatus in western North America. *J. Fish Biol.* **75**, 2075-2081. doi:10.1111/j.1095-8649.2009.02418.x

Zhang, J., Song, Q., Cregan, P. B., Nelson, R. L., Wang, X., Wu, J. and Jiang, G.-L. (2015). Genome-wide association study for flowering time, maturity dates and plant height in early maturing soybean (Glycine max) germplasm. *BMC Genomics* **16**, 217. doi:10.1186/s12864-015-1441-4

Ziuganov, V. V., Golovatjuk, G. J., Savvaitova, K. A. and Bugaev, V. F. (1987). Genetically isolated sympatric forms of threespine stickleback, Gasterosteus aculeatus, in Lake Azabachije (Kamchatka-peninsula, USSR). *Environ. Biol. Fishes* **18**, 241-247. doi:10.1007/BF00004877