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## Population- and sex-specific divergence in growth patterns between two ninespine stickleback (*Pungitius pungitius* L) populations

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### ABSTRACT

**Background:** Growth rate is an important life-history trait that often shows sex- and population-specific differentiation in many organisms. Yet the relative contributions of additive genetic, non-additive genetic, environmental, and maternal effects underlying these differences remain largely unknown, especially in wild animal populations.

**Goal:** To determine the relative contributions of additive genetic, non-additive genetic, and environmental effects underlying population differences in growth rate between two stickleback populations differing markedly in their body size.

**Organism:** Ninespine stickleback (*Pungitius pungitius*).

**Methods:** We crossed two phenotypically and genetically distinct populations to produce ‘pure’ marine (Hel-Hel; small sized), ‘pure’ pond (Pyö-Pyö; large sized), and ‘hybrid’ (Hel-Pyö and Pyö-Hel) offspring. We reared them in standardized common garden settings until maturation.

**Results:** Analyses of Von Bertalanffy growth curve parameters revealed that sexes and cross-types differed in their intrinsic growth rates ( $k$ ) and asymptotic sizes ( $L_{\infty}$ ). In general, males and marine fish (Hel-Hel) had higher  $k$  and smaller  $L_{\infty}$  than females and fish from the pond (Pyö-Pyö). Fish from ‘hybrid’ crosses exhibited  $k$  and  $L_{\infty}$  intermediate to the ‘pure’ crosses, but were more similar in both respects to the pure marine than to the pure pond fish. Thus population differentiation in  $k$  and  $L_{\infty}$  has a genetic basis, but additive genetic effects do not explain all the observed differences.  $k$  and  $L_{\infty}$  were negatively correlated within three cross-types (both ‘hybrids’ and Pyö-Pyö): low intrinsic growth rates were associated with increased asymptotic size.  $k$  and  $L_{\infty}$  were not correlated within the Hel-Hel cross: high intrinsic growth rate was not directly associated with reduced asymptotic size. Neither  $k$  nor  $L_{\infty}$  predicted the age at maturation in Hel-Hel fish, and only poorly so in Pyö-Pyö fish.

**Conclusion:** We discovered genetically based population differentiation in key growth-related life-history traits, but little or no evidence for a role of intrinsic growth rate or asymptotic size in determining the timing of maturation in ninespine stickleback.

**Keywords:** asymptotic length, common garden, growth rate, maturation, *Pungitius pungitius*, von Bertalanffy.

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## INTRODUCTION

Growth rate defines the relationship between size and age of an individual, and size in turn influences both the individual's survival probability (e.g. Gross, 1981; McGraw and Wulff, 1983; Werner, 1988) and fecundity (e.g. McGraw and Wulff, 1983; Shine, 1988; Aarssen and Clauss, 1992). Therefore, all else being equal, one would expect organisms to grow as fast as possible to achieve the benefits of large size (e.g. Ricklefs, 1969). This at least until the age at first reproduction because the onset of maturation causes an individual to channel energy away from growth to reproduction with a concomitant decrease, or even cessation, of growth (Chapman *et al.*, 2011). Consequently, the onset of maturation can influence both the individual's size at maturation, as well as its fecundity (Roff, 1992; Stearns, 1992, 2000; Shimada *et al.*, 2011; Williams *et al.*, 2012). Thus, by delaying maturation, an individual can prolong its growth period, reach a larger adult size (e.g. Shimada *et al.*, 2011; Gambling and Reimchen, 2012; Herczeg *et al.*, 2012), and increase its fecundity (e.g. Shine, 1988; Stearns, 1992; Angilletta *et al.*, 2004). In contrast, early maturation can reduce adult size and fecundity either because of a shortened growth period (Roff, 1992; Stearns, 1992, 2000; Shimada *et al.*, 2011) or the cost of rapid growth itself (e.g. Madsen, 1983; Conover and Present, 1990; Stearns, 1992; Partridge and Coyne, 1997; Post *et al.*, 1999; Brito and Rebelo, 2003; Herczeg *et al.*, 2012). Therefore, the onset of maturation can be an important source of variation in growth dynamics both within and between populations.

Differences in the onset of maturation can also underlie differences between the sexes in growth and size at maturity. In general, females mature later than males chiefly because sperm production generally requires less energy than egg production (Charnov, 1982; Fleming, 1996). For example, in Atlantic salmon, females invest approximately 25% of their weight into gonads, whereas anadromous males invest approximately 3–6% and male parr invest approximately 9% of their weight into gonads (Fleming, 1996). This shows that females can invest over six times as much energy into gonads as males. Furthermore, egg production is not only more energy demanding than sperm production, but females may also gain fecundity with age and size at a higher rate than males (e.g. Shine, 1988; Angilletta *et al.*, 2004). Although sperm production requires less energy than egg production, males may suffer from energetic costs of behaviour associated with successful reproduction such as territoriality, nest building, and parental care (e.g. Östlund-Nilsson *et al.*, 2007).

However, the causality in these relationships is often unclear: while the onset of maturation can influence growth, growth (rates) can affect the onset of maturation (e.g. Morita and Fukuwaka, 2006; Kuparinen *et al.*, 2011). Indeed, there has been much discussion about the extent to which changes in timing of maturation reflect the evolution of timing of maturation itself, and the extent to which the changes in timing of maturation are correlated responses to plastic or evolutionary changes in growth (Kinnison *et al.*, 2011; Borrell, 2013). These are all issues that may have important implications for our understanding of how fisheries-induced selection may influence the evolution of fish life histories (e.g. Law, 2000, 2007; Olsen *et al.*, 2004; Borrell, 2013). For instance, the influence of growth rate on timing of maturation may simply reflect phenotypic plasticity in response to fishing, which reduces stock biomass and relaxes competition for resources and thus accelerates growth for the remaining fish (e.g. Lorenzen and Enberg, 2002; Dieckmann and Heino, 2007). Alternatively, if growth rate and age at maturation are genetically correlated, faster-growing fish can evolve to mature at an earlier age than slower-growing fish (e.g. Alm, 1959; Roff, 1982; Hutchings, 1993; Rijnsdorp, 1993).

In previous research, we uncovered marked and genetically based differences in both size and age at maturity between two Fennoscandian ninespine stickleback (*Pungitius pungitius*) populations (Ab Ghani *et al.*, 2012, 2013). Fish from the marine population mature at earlier age

and smaller size than fish from the pond population, and these differences appear to have a genetic basis. However, while the body size divergence was found to be mostly due to additive genetic effects (Ab Ghani *et al.*, 2012), early maturation seemed to be a dominant trait: the mean age at maturity in reciprocal crosses between marine and pond populations was more similar to that in pure marine crosses than to that in pure pond crosses (Ab Ghani *et al.*, 2013). We also found that males matured earlier and at smaller size than females (Ab Ghani *et al.*, 2013). These findings, together with evidence from other empirical (Herczeg *et al.*, 2009a, 2010; Shimada *et al.*, 2011; Välimäki and Herczeg, 2012) and theoretical (Aikio *et al.*, 2013) studies in this system suggest that late maturation at large size in the pond population is explained by adaptation to a pond environment free of piscine predators. Although previous studies have documented divergence in growth rates among these populations [and sexes (Shimada *et al.*, 2011; Herczeg *et al.*, 2012; Välimäki and Herczeg, 2012; Aikio *et al.*, 2013)], all of them were based on intra-population crosses where the influence of maternal and non-additive genetic effects on the expression of focal traits cannot be controlled. Here, we took a step forward by producing inter-population crosses where the relative importance of additive and non-additive genetic effects, as well as cross-generational maternal/environmental effects, on variation in growth curve parameters between populations and sexes can be explored. Given that maternal and cross-generational effects on growth parameters are commonplace (e.g. Rossiter, 1996; Green 2008; Salinas and Munch, 2012), analyses of inter-population crosses provide means to elucidate their potential impact on growth parameters. Similarly, the significance of growth rate as a predictor of the onset of maturation has previously been investigated only in the marine population (Kuparinen *et al.*, 2011), and the question of whether fast growth translates into early maturation also applies to the pond population (and both sexes) remains unanswered. Previous studies using the same breeding design with the same fish as in the present study have revealed that there are genetically based differences in both size at age (Ab Ghani *et al.*, 2012) and age at maturation (Ab Ghani *et al.*, 2013) between marine and pond populations. Because growth is an underlying mechanism through which differences in maturation and in body size at different life-stages arise (e.g. Alm, 1959), a full understanding of the processes leading to differentiation in size and age at maturation requires that we understand also the mechanisms underlying differentiation in growth patterns. Furthermore, understanding differentiation in growth allows for projections for the asymptotic body size, which is a pivotal fitness component.

The aim of this study was to explore the genetic basis of growth curve parameters – intrinsic growth rate ( $k$ ) and asymptotic length ( $L_{\infty}$ ) as estimated by the method of von Bertalanffy (1938) – in a reciprocal common garden cross experiment using laboratory-reared  $F_1$ -generation individuals from two Fennoscandian ninespine stickleback populations known to differ genetically in their size and age at maturation. One of the populations was a small-sized and early maturing marine population (Helsinki = Hel), and the other a large-sized and late maturing pond population (Pyöreälampi = Pyö). We hypothesized that if variation in growth between the populations and sexes is due to additive genetic effects, the ‘pure’ crosses (Hel-Hel and Pyö-Pyö) will differ in their growth parameters, while the ‘hybrids’ (Hel-Pyö and Pyö-Hel) will be intermediate in their growth compared with the ‘pure’ crosses (cf. Wright, 1978; Nestor *et al.*, 2005). In the case that non-additive genetic or maternal effects are influential, the ‘hybrids’ should deviate from the expected intermediacy between the pure lines. For instance, with simple dominance, individuals from both ‘hybrid’ crosses are expected to deviate towards the mean of one of the ‘pure’ crosses possessing the dominant allele(s). Similarly, if maternal effects are present, individuals from both ‘hybrid’

crosses are expected to deviate from the intermediacy towards their mothers' 'pure' line means (cf. Wright, 1978; Nestor *et al.*, 2005). In addition, to address the controversies in the literature regarding fisheries-induced evolution (e.g. Dieckmann and Heino, 2007), we addressed the question of whether the variation in intrinsic growth rates is linked to the variation in timing of maturation.

## MATERIALS AND METHODS

### Sampling and breeding

The parental generation of ninespine sticklebacks was collected using seine nets and minnow traps before or during the early phase of the reproductive season (late May to mid June 2010) from two geographically separated ( $\approx 900$  km) and genetically isolated (Shikano *et al.*, 2010) populations. The two populations were a marine population (the Baltic Sea at Helsinki;  $60^{\circ}12'09''\text{N}$ ,  $25^{\circ}10'58''\text{E}$ ) and a pond population (Pyöreälampi;  $66^{\circ}15'40''\text{N}$ ,  $29^{\circ}26'00''\text{E}$ ). The marine population is from a shallow coastal habitat of the Baltic Sea with low salinity [ $\approx 6.0$  psu (Shimada *et al.*, 2011)]. Here, the ninespine sticklebacks co-exist with interspecific competitors such as threespine stickleback (*Gasterosteus aculeatus*) and piscine predators such as pike (*Esox lucius*) and perch (*Perca fluviatilis*). The marine ninespine stickleback are 'normal'-sized fish with a lifespan of about 3 years (Herczeg *et al.*, 2009a) and are able to reproduce one year after hatching (Herczeg *et al.*, 2009a; Shimada *et al.*, 2011; Ab Ghani *et al.*, 2013). The Pyöreälampi population is from a freshwater pond with a surface area of less than 5 ha. Here, the ninespine stickleback co-exist with introduced whitefish (*Coregonus lavaretus*), which are currently either extinct or present at very low density. The pond ninespine stickleback are 'giant'-sized (Herczeg *et al.*, 2009a, 2010), can live at least up to the age of 7 years (Herczeg *et al.*, 2009a), and exhibit delayed maturity (Herczeg *et al.*, 2009a; Shimada *et al.*, 2011; Ab Ghani *et al.*, 2013).

Artificial fertilizations among randomly chosen males and females from the two populations were conducted from 14 to 20 June 2010 to produce a total of 40 full-sib families of four different cross-types (i.e. 10 full-sib families per cross-type): two 'pure' crosses were created by crossing marine males with marine females (hereafter Hel-Hel) or by crossing pond males with pond females (hereafter Pyö-Pyö). The two 'hybrid' crosses were created by crossing either pond males with marine females (hereafter Pyö-Hel) or by crossing marine males with pond females (hereafter Hel-Pyö). Each individual male and female were used only to produce one cross. The artificial fertilizations were done by gently squeezing out the eggs from ripe females and mixing sperm solution with the eggs. The sperm solution was obtained by mincing the testicles of over-anaesthetized males with MS222 (tricaine methanesulphonate). Owing to logistic constraints, artificial fertilizations were made both at the sampling site and in the laboratory. Thus, the conditions for the fertilized eggs on the first two days were not identical, but this did not influence the subsequent egg size (Ab Ghani *et al.*, 2012). Clutches were kept in Petri dishes until hatching and water was changed twice a day. All developing eggs were regularly checked under dissecting microscope and any dead or unfertilized eggs removed.

When the larvae started to swim freely (about 7 days after hatching), we randomly isolated five larvae per family from each cross-type into 1.4-litre tanks in four Allentown Zebrafish Rack Systems (hereafter rack; Aquaneering Inc., San Diego, USA). Each rack contained 100 of the 1.4-litre tanks in a closed water circulation system equipped with physical, biological, and UV filters. White plastic panels were placed between the tanks to

block any visual contact between individual fish. All fish were reared in freshwater (salinity 0 psu) and fed *ad libitum* with live brine shrimp (*Artemia* sp.) nauplii for the first 2 months and with frozen bloodworms thereafter. For 299 days after hatching (hereafter DAHs), the water temperature was set to 17°C and the photoperiod to 14/10 h light/dark. At 300 DAHs, over a 2 week period, all fish were put into artificial hibernation, by gradually shifting the photoperiod to 0/24 h light/dark, and gradually lowering the water temperature to 4°C. After 30 days under the artificial hibernation conditions, again over a 2 week period, we gradually increased the water temperature back to 17°C and changed the photoperiod to 24/0 h light/dark to stimulate testis and ovary development (cf. Baggerman, 1985). All fish were then maintained at a temperature 17°C and a photoperiod of 24/0 h light/dark for 97 days. After 455 DAHs, all Hel-Hel fish, 98% of Hel-Pyö fish, 96% of Pyö-Hel fish, and 39% of Pyö-Pyö fish had matured (Ab Ghani *et al.*, 2013). Because only 39% of Pyö-Pyö fish had matured, we subjected all the fish to a second artificial hibernation, following the above protocol. We suspected that the 61% of Pyö-Pyö fish that had not matured were females, because females in this species mature later than males (e.g. Kuparinen *et al.*, 2011; Shimada *et al.*, 2011), and that a repeated artificial hibernation period was needed to stimulate their ovary development. Since the beginning of sexual maturation is influenced by many factors, including temperature (Garrard *et al.*, 1974; Marion, 1982; Kuparinen *et al.*, 2011) and photoperiod (Baggerman, 1985; Taranger *et al.*, 1999), we took every precaution to standardize the conditions in which the fish were raised so that any differences or similarities in age at maturation among different cross-types could be attributed to genetic factors and not environmental conditions.

### Data collection

The final data set of growth trajectories consisted of 192 individuals: 84 males (22 Hel-Hel, 16 Hel-Pyö, 27 Pyö-Hel, and 19 Pyö-Pyö) and 108 females (27 Hel-Hel, 34 Hel-Pyö, 23 Pyö-Hel, and 24 Pyö-Pyö) with a maximum of 15 standard length measurements (i.e. the length from the tip of the lower jaw to the base of the caudal peduncle) from photographs taken at 30, 60, 90, 120, 150, 180, 210, 240, 270, 330, 360, 390, 420, 480, and 510 DAHs to the closest 0.01 mm using the program tpsDig2 (Rohlf, 2006). All fish were photographed using a digital camera (Nikon D60), and a ruler was used as a size reference in each photograph. Eight individuals were removed from the data set: four individuals that died early in the experiment, and another four individuals that were clearly malformed as judged from their von Bertalanffy (hereafter VB) growth curve: no reliable information on their growth trajectories could be obtained. In addition, we recorded the first age at maturation (hereafter age at maturation) of individual fish by visually inspecting them every day starting from the day when the first artificial hibernation ended. The age at maturation data used here are the same as those reported in Ab Ghani *et al.* (2013). After 8 weeks of observations, a total of 156 individuals had matured – 80 males (21 Hel-Hel, 15 Hel-Pyö, 26 Pyö-Hel, and 18 Pyö-Pyö) and 76 females (23 Hel-Hel, 32 Hel-Pyö, and 21 Pyö-Hel), while 31 individuals remained immature – 2 males (both Pyö-Pyö) and 29 females (1 Hel-Pyö, 2 Pyö-Hel, and 26 Pyö-Pyö). Non-maturing individuals were not segregated into particular families; rather, maturation was observed in every family – for instance, all families were represented in the sample of the 26 immature Pyö-Pyö females. Immature individuals were sexed using the molecular sexing method of Shikano *et al.* (2011), as described in Ab Ghani *et al.* (2013).

### Statistical analyses

Each individual growth trajectory (based on 15 measurements, see above) was summarized through a VB growth curve as follows:

$$l(t) = L_{\infty} - (L_{\infty} - L_0)e^{-kt} \quad (1)$$

where  $l(t)$  is the size of an individual at age  $t$ ,  $L_0$  is average size at  $t = 0$ ,  $L_{\infty}$  is the asymptotic size, and  $k$  is the intrinsic growth rate, i.e. the rate at which  $L_{\infty}$  is reached (von Bertalanffy, 1938). The VB curves were fitted to the length-at-age measurements through non-linear least-squares regressions, separately for males and females, as growth in ninespine sticklebacks is known to differ between the sexes (Kuparinen *et al.*, 2011). Thus, the estimated parameters of the VB curve ( $L_{\infty}$ ,  $L_0$ , and  $k$ ) were treated as free model parameters. In further analyses, we focused on  $L_{\infty}$  and  $k$ , as these mostly determine the growth trajectory of the individual, while  $L_0$  has little biological importance.

The VB growth parameters  $L_{\infty}$  and  $k$  (log-transformed for the normality of residuals) were analysed using linear mixed effects (LME) modelling, to determine whether they differed significantly between populations. Models were again fitted separately for males and females. In each LME model, either  $L_{\infty}$  or log-transformed  $k$  was considered as a response variable, cross-type (i.e. Hel-Hel, Pyö-Hel, Hel-Pyö, Pyö-Pyö) as a fixed effect, and family as a random effect.

Next, using LME, we also investigated whether variation in individual VB growth parameters could influence the difference in maturation within and between populations. To this end, age at maturation was considered as a response variable,  $L_{\infty}$ , log-transformed  $k$ , and cross-type as fixed effects, and family as a random effect. Both sexes were again modelled separately, as growth influences timing of maturation in males and females differently (Kuparinen *et al.*, 2011). For all LME models, stepwise model reductions were performed by comparing likelihood ratios ( $LR$ ), as suggested by Crawley (2007). Residuals were examined for normality and homogeneity. All analyses were performed using R v.2.10.1 (R Development Core Team, 2009).

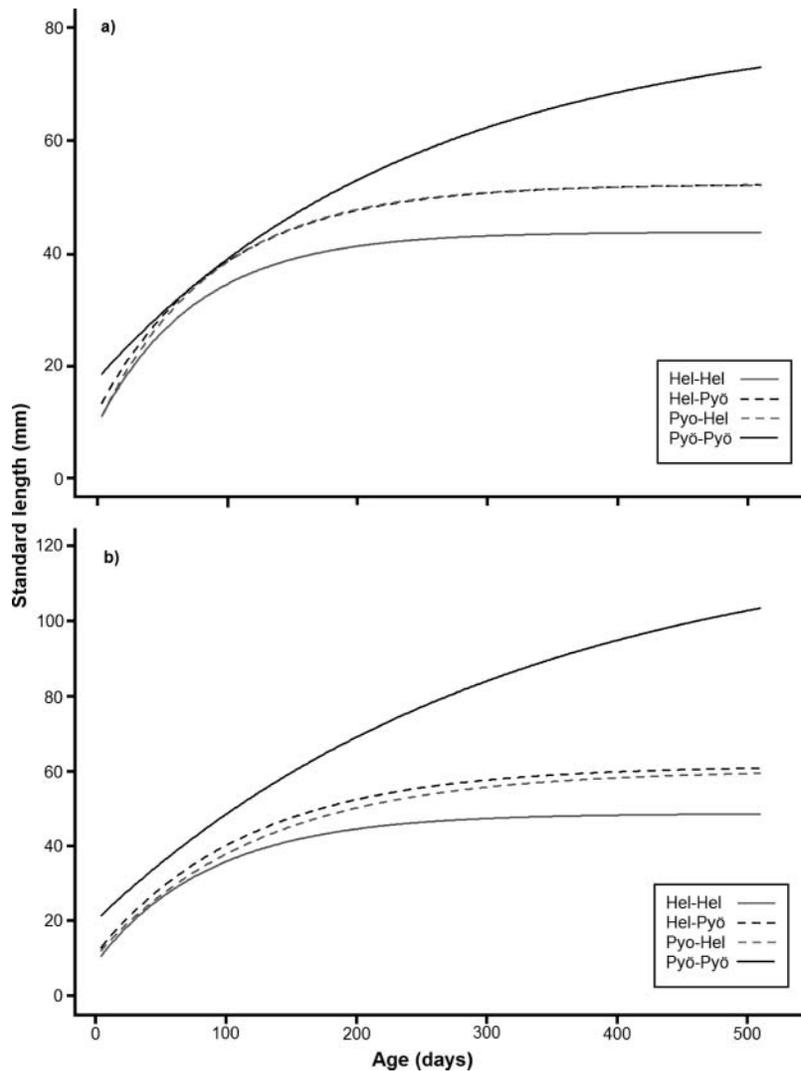
## RESULTS

### Fitting the growth curves

In general, the VB curves for both males and females fitted very well to the individual growth trajectories with a coefficient of determination ( $R^2$ -values) of 0.882–0.998 for males and 0.969–0.997 for females. Thus, the three VB parameters can be considered to adequately summarize the growth of each individual. The cross-type and sex-specific mean growth curves are shown in Fig. 1. The parameters  $L_{\infty}$  and log-transformed  $k$  were not significantly correlated for males or females of the marine population (Hel-Hel), but they were strongly negatively correlated for both males and females in all the other cross-types (Table 1).

### Analysis of growth curve parameters

Both males and females from the marine population (Hel-Hel) had significantly faster intrinsic growth rates and smaller asymptotic sizes than males and females from the pond



**Fig. 1.** Mean von Bertalanffy growth trajectories of four cross-types of ninespine sticklebacks. Trajectories were fitted through non-linear least-squares regression separately for (a) males and (b) females. Hel = Helsinki, Pyö = Pyöreälampi. The first abbreviation denotes the origin of father, the second the origin of mother.

population (Pyö-Pyö; Fig. 1). Intrinsic growth rates and asymptotic sizes were very similar in the two 'hybrid' crosses (Hel-Pyö and Pyö-Hel) in both males and females, and intermediate when compared with the 'pure' crosses (Hel-Hel and Pyö-Pyö; Fig. 1).

LME analysis confirmed that in males  $L_{\infty}$  and log-transformed  $k$  were not significantly different between the 'hybrid' cross-types ( $L_{\infty}$ : likelihood ratio ( $LR$ ) = 0.001, d.f. = 5,  $P$  = 0.979; log-transformed  $k$ :  $LR$  = 0.440, d.f. = 5,  $P$  = 0.507), and thus the factor levels Hel-Pyö and Pyö-Hel were combined (hereafter 'hybrids'). Similarly, in females  $L_{\infty}$  and log-transformed  $k$  were not significantly different between the 'hybrid' cross-types

**Table 1.** The relationship between the von Bertalanffy parameters (log-transformed  $k$  and  $L_{\infty}$ ) of four cross-types of ninespine sticklebacks

Sex	Cross-type	$r$	d.f.	$P$
Male	Hel-Hel	0.329	22	0.135
	Hel-Pyö	0.572	16	0.020
	Pyö-Hel	0.596	27	0.001
	Pyö-Pyö	0.837	19	< 0.0001
Female	Hel-Hel	0.235	27	0.239
	Hel-Pyö	0.827	34	< 0.0001
	Pyö-Hel	0.535	23	0.009
	Pyö-Pyö	0.740	22	< 0.0001

**Table 2.** The LME results of the growth parameters ( $L_{\infty}$  and  $k$ ) of four cross-types of ninespine sticklebacks

Response	Explanatory variables	Parameter estimate ( $\pm$ S.E.)	d.f.	$t$
$L_{\infty}$ (males) (family VC = < 0.01%) <sup>a</sup>	Intercept	43.7909 ( $\pm$ 1.8978)	45	23.074**
	'Hybrids'	8.5110 ( $\pm$ 2.3334)	36	3.647****
	Pyö-Pyö	37.0581 ( $\pm$ 2.7879)	36	13.292**
log( $k$ ) (males) (family VC = < 0.01%) <sup>a</sup>	Intercept	-4.3456 ( $\pm$ 0.0615)	45	-70.658***
	'Hybrids'	-0.1798 ( $\pm$ 0.0756)	36	-2.378*
	Pyö-Pyö	-1.2370 ( $\pm$ 0.0903)	36	-13.692***
$L_{\infty}$ (females) (family VC = 25.3%) <sup>a</sup>	Intercept	48.6118 ( $\pm$ 6.2992)	68	7.717***
	'Hybrids'	12.3744 ( $\pm$ 7.6958)	37	1.608 <sup>ns</sup>
	Pyö-Pyö	74.5376 ( $\pm$ 9.0321)	37	8.252***
log( $k$ ) (females) (family VC = 13.3%) <sup>a</sup>	Intercept	-4.4953 ( $\pm$ 0.1005)	68	-44.748***
	'Hybrids'	-0.3338 ( $\pm$ 0.1225)	37	-2.725**
	Pyö-Pyö	-1.6560 ( $\pm$ 0.1449)	37	-11.429***

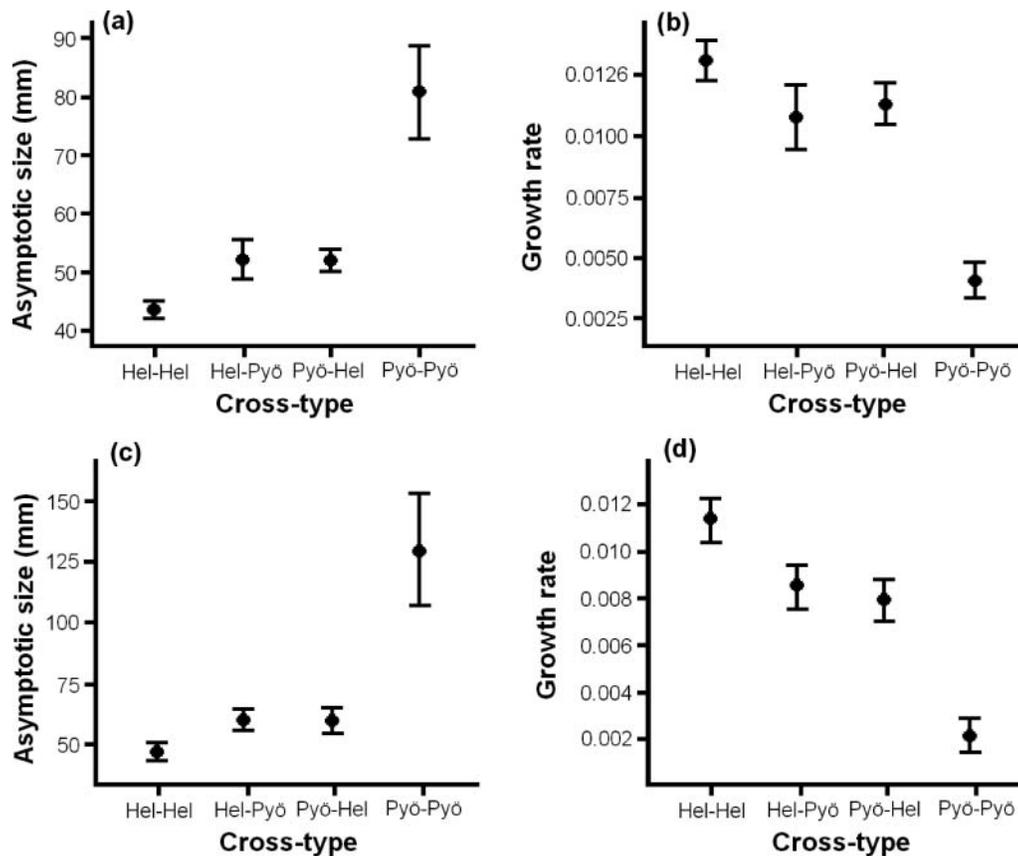
Note: Intercept refers to the Hel-Hel cross-type and 'Hybrids' refers to the two reciprocal inter-population crosses (Hel-Pyö and Pyö-Hel cross-types). Hel = Helsinki, Pyö = Pyöreälampi. The first abbreviation denotes the origin of father, the second the origin of mother.

<sup>a</sup> Variance component (VC) of family within cross-type.

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

( $L_{\infty}$ :  $LR = 0.019$ , d.f. = 5,  $P = 0.890$ ; log-transformed  $k$ :  $LR = 0.148$ , d.f. = 5,  $P = 0.701$ ). Thus, the final sex-specific LME models of the growth parameters were fitted with either  $L_{\infty}$  or log-transformed  $k$  as a response variable, three levels of cross-types as a fixed effect (Hel-Hel, 'hybrids', Pyö-Pyö), and family as a random effect (Table 2).

The final LME model for males revealed that Pyö-Pyö fish differed significantly from the other cross-types ('hybrids' and Hel-Hel) in their  $L_{\infty}$  and log-transformed  $k$



**Fig. 2.** Mean ( $\pm$  95% CI) intrinsic growth rates (log-transformed  $k$ ) and asymptotic sizes ( $L_{\infty}$ ) of male and female ninespine sticklebacks as estimated from VB curves fitted for individual data. (a)  $L_{\infty}$  for males, (b) log-transformed  $k$  for males, (c)  $L_{\infty}$  for females, and (d) log-transformed  $k$  for females. Hel = Helsinki, Pyö = Pyöreälampi. The first abbreviation denotes the origin of father, the second the origin of mother.

parameters (Fig. 2). The family variance component had a negligible effect ( $< 0.01\%$  for both parameters). The final LME model for females revealed that Pyö-Pyö differed significantly from the other cross-types ('hybrids' and Hel-Hel) in their  $L_{\infty}$  and log-transformed  $k$  parameters with moderate amounts of variation associated with the random effect of family (variance components for  $L_{\infty}$  and log-transformed  $k$  = 25.3% and 13.3% respectively; Fig. 2).

#### The effect of growth and asymptotic length on timing of maturation

In males, the age at maturation was similar among Hel-Hel, Pyö-Hel, and Hel-Pyö cross-types (combined Pyö-Hel and Hel-Pyö:  $LR = 1.115$ , d.f. = 7,  $P = 0.291$ ; joining 'hybrids' and Hel-Hel:  $LR = 0.099$ , d.f. = 6,  $P = 0.753$ ), but these cross-types differed significantly from Pyö-Pyö (Table 3). Age at maturation was not related to VB growth parameters

**Table 3.** The LME results of age at maturation of four cross-types of ninespine sticklebacks

Response variable	Explanatory variables	Parameter estimate ( $\pm$ S.E.)	d.f.	<i>t</i>
Age at maturation (males) (family variance = 5.02%) <sup>a</sup>	Intercept (Hel-Hel + 'Hybrids')	361.30 ( $\pm$ 1.06)	41	342.24****
	Pyö-Pyö	46.56 ( $\pm$ 2.26)	36	20.59****
Age at maturation (females) (family variance = < 0.01%) <sup>a</sup>	Intercept	380.70 ( $\pm$ 2.77)	48	137.55****
	'Hybrids'	10.38 ( $\pm$ 3.31)	26	3.13**

Note: 'Hybrids' refers to the two reciprocal inter-population crosses (Hel-Pyö and Pyö-Hel cross-types). Hel = Helsinki, Pyö = Pyöreälampi. The first abbreviation denotes the origin of father, the second the origin of mother.

<sup>a</sup> Variance component of family within cross-type.

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

( $L_{\infty}$ :  $LR = 0.108$ , d.f. = 5,  $P = 0.742$ ; log-transformed  $k$ :  $LR = 3.528$ , d.f. = 4,  $P = 0.060$ ). The effect of family variance remained small (5.02%; Table 3).

For females, the LME model was based on three cross-types only (Hel-Hel, Hel-Pyö, Pyö-Hel) because none of the Pyö-Pyö females had matured by the last observation day (510 DAH). Age at maturation did not differ significantly between the 'hybrid' cross-types, and thus the Pyö-Hel and Hel-Pyö crosses were combined ( $LR = 0.953$ , d.f. = 6,  $P = 0.329$ ). Age at maturation was not related to the VB growth parameters ( $L_{\infty}$ :  $LR = 2.044$ , d.f. = 4,  $P = 0.153$ ; log-transformed  $k$ :  $LR = 0.242$ , d.f. = 5,  $P = 0.623$ ). Again, the effect of family variance was negligible (< 0.01%; Table 3).

## DISCUSSION

The results of the present study show that variation in the intrinsic growth rate ( $k$ ) – the relative rate at which an individual reaches asymptotic size – and the asymptotic size ( $L_{\infty}$ ) between populations and sexes of ninespine sticklebacks are at least partly attributable to genetic effects, but the mode of gene action underlying the divergence appears to be mainly dominant. Males and marine fish (Hel-Hel) grew significantly faster and matured at a smaller size than females and pond fish (Pyö-Pyö). However, growth rate and the asymptotic size of hybrid fish (Hel-Pyö and Pyö-Hel) were intermediate between the marine and the pond crosses, with a strong tendency for the hybrids to be more similar to the pure marine than to the pure pond fish in both sexes. No evidence was found for an association between the variation in intrinsic growth rate and the variation in asymptotic size in the marine population, and only a weak association was found in the pond and the hybrid fish. Similarly, the onset of maturation was not predictable from asymptotic size or intrinsic growth rates in either of the sexes. We now discuss these findings and their implications for our understanding of growth rate differentiation among populations, as well as how variation in growth rates translates into – or is associated with – variation in final size and timing of maturation within and among different populations.

The divergence in growth rate and body size between marine and pond ninespine stickleback populations has been suggested to have arisen as an adaptive response to the absence of piscine predation, reduced interspecific and increased intraspecific competition in the

pond environment (e.g. Herczeg *et al.*, 2012; Aikio *et al.*, 2013). The results of this study support these adaptive explanations by showing that the growth rate differences between pond and marine populations have a genetic basis. The results are clearly incompatible with the idea that the influence of population-specific maternal effects would be important determinants of growth rate variation in this system: there was no tendency whatsoever for the growth rates of the hybrids to resemble those of the pure crosses involving their maternal parents. However, the observation that in both reciprocal hybrid crosses the growth rates were more similar to the growth rates of the pure marine than the pure pond fish suggests that there are strong dominance effects (see Fig. 2). In spite of this, the two reciprocal hybrid crosses differed statistically from them in overall growth rates (Table 2). It would have been informative to have estimated the dominance contributions free of any potential biases by using back crosses (e.g. Lynch and Walsh, 1998), but this option was not available to us because we have been unable to raise the F<sub>1</sub>-generation pond females to a breeding condition in spite of numerous attempts to this effect (N.I. Ab Ghani *et al.*, unpublished results). Nevertheless, Ab Ghani *et al.* (2013) reported very similar results and inferences as in the present study when analysing timing of maturation in this very same cross. Hence, it appears that both high growth rates and early timing of maturation show directional dominance towards the phenotypes of the marine parents. To this end, the results are in line with the interpretation that high growth rates and early maturation are features that have been selected for in the marine environment (Ab Ghani *et al.*, 2012; Herczeg *et al.*, 2012; Aikio *et al.*, 2013).

Across the four different cross-types, higher growth rates were associated with smaller asymptotic size: fish from the crosses involving one or more marine parents grew fast and matured early at a small size. In contrast, pure pond fish grew at a slower pace, matured late and at a large size. When looking for associations within the crosses at the individual level, growth rates and asymptotic sizes were negatively correlated in both hybrid and pond fish, showing that the associations observed at the level of cross-types were also present at the individual level. However, these correlations were relatively weak, and they were not observed within the pure marine fish. Nevertheless, these patterns are consistent with earlier analyses of growth rates within (two) pond and (two) marine populations of nine-spine sticklebacks, which again showed negative correlations between intrinsic growth rate and asymptotic size (Kuparinen *et al.*, 2011; Herczeg *et al.*, 2012). In fact, this pattern appears to be fairly general among ectothermic animals (Berrigan and Charnov, 1994). Hence, in this respect our results conform to earlier findings, with the exception that population divergence in these patterns is genetically, rather than environmentally (or maternally), based.

In an earlier experiment done with Helsinki fish, it was found that the age at maturation was predictable – or at least significantly positively correlated with – the intrinsic growth rate in both sexes, and also with asymptotic size in males (Kuparinen *et al.*, 2011). In this study, no such effects were observed. This discrepancy in the results of the two studies could be due to simple statistical power issues stemming from relatively small sample sizes used in this study. However, the sample sizes in this study (192 individuals for *k* vs. 156 individuals for age at maturation analyses) and the earlier study (109 individuals) were not very different, and effects in the earlier study were also relatively weak (Kuparinen *et al.*, 2011). Hence, while it would be perhaps premature to conclude that there is no association between age at maturation and the growth parameters (i.e. intrinsic growth rate and asymptotic size), it appears that the predictive power of any such relationship is likely to be limited in these fish. In other words, factors other than those related to the intrinsic variation in individual growth trajectories, such as food availability and predation might be more important

determinants of timing of maturation. Because all the experimental fish were raised in a common garden setting, the possibility of any ecological constraints – such as variation in predation, interspecific and conspecific competition, parasitism and resource availability – having influenced growth and maturation can be largely excluded. Also, we took every precaution to standardize the conditions the fish were raised in: (1) fish were fed *ad libitum*, so that it was unlikely that the fish underwent resource limitation; (2) fish were kept individually to avoid any possible adverse social effects or competition with conspecifics (cf. Herczeg *et al.*, 2009b); and (3) the temperature was set close to the upper margin of the reported preference temperature range of the species (Lanche *et al.*, 1987), so that our fish experienced an optimum temperature for growth. Hence, the minimized environmental heterogeneity in our experimental setting might provide one explanation for the lack of association between age at maturation and intrinsic growth rates. Similarly, it is possible that we may have produced such a high growth that we were assessing these traits outside their natural bounds, which could explain the lack of association. By inference, the presence of environmental heterogeneity – as would be the case in natural settings – which influences growth rates, might also influence timing of maturation, creating correlations between these variables. However, Shimada *et al.* (2011) reported a strong positive genetic correlation ( $r_g = 0.87$ ) between age and size at maturity, suggesting that the two traits are genetically strongly linked. Similarly, the patterns of covariation between intrinsic growth rates (and asymptotic size) and age at maturity (Ab Ghani *et al.*, 2012) across the four cross-types is strong: pond fish mature late at a large size (and have low  $k$ ), whereas the fish from the other three cross-types mature early at a small or an intermediate size (and have high  $k$ ). Hence, the evidence for associations between intrinsic growth rate, asymptotic size, and age at maturation is conflicting, or at least, varies depending on whether they are examined within or across crosses. Whatever the explanation for these different results, one thing is clear: pond and marine populations are strongly genetically divergent in both their intrinsic growth rate (this study) and the age at maturity (Ab Ghani *et al.*, 2012), and this divergence shows clear signs of dominance associated with genes originating from the marine population.

Finally, it should be noted that there is potentially an important difference between this study and those that considered growth effects on age at maturity in other taxa. In this study, we looked at timing of maturation within a reproductive season, whereas many other studies have typically looked at timing of maturation at the level of year irrespective of the exact day of maturation within those years (e.g. Madsen, 1983; Post *et al.*, 1999; Brito and Rebelo, 2003). Thus the present and previous studies might have looked at rather different processes, where the roles of growth and/or body size may also have differed. With these differences in mind, the present study nonetheless provides novel insights into the genetic architecture of maturation and how it may evolve. If the alleles associated with late maturation and large body size are recessive, evolution towards these phenotypes is much slower than towards the small body size and early maturation that are expressed by dominant alleles. This is in line with many observations in natural fish populations, where age and body size have been seen to decline with size-selective harvesting (Kuparinen and Merilä, 2007; Fenberg and Roy, 2008). From a fisheries management perspective, the present results also suggest that it might not be a very efficient practice to try to increase fish body size and age at maturation by setting a maximum size limit for allowable catch (Arlinghaus *et al.*, 2009).

## CONCLUSION

The differences in growth rates between the marine and the pond populations of ninespine sticklebacks appear to be attributable to genetic rather than maternal effects. These genetic effects seem to have a strong dominance component, so that the genetic factors determining growth rates in the marine environment overshadow the influence of the factors prevalent in the pond environment. Furthermore, it was found that intrinsic growth rates and asymptotic size were poor predictors of age at maturation, suggesting that this key life-history event is not predictable from knowledge of individual growth parameters in the ninespine stickleback, not least in the pond fish (cf. Kuparinen *et al.*, 2011). Further studies identifying genomic region(s) and genetic factor(s) responsible for the growth rate differentiation in ninespine sticklebacks would provide an obvious next step towards a better understanding of the proximate determinants of growth rate variation in this species.

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