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Classification	Biological Sciences
Title	Large single-locus effects for maturation timing are mediated via condition variation in Atlantic salmon
Authors	Paul V Debes[a], Nikolai Piavchenko[a], Annukka Ruokolainen[a], Outi Ovaskainen[a], Jacqueline E Moustakas-Verho[a], Noora Parre[a] Tutku Aykanat[a], Jaakko Erkinaro[b], Craig R Primmer[a]
Author Affiliations	[a]Organismal & Evolutionary Biology Research Program / Institute of Biotechnology, University of Helsinki, Viikinkaari 9, 00014, Finland; [b]Natural Resources Institute Finland (Luke), Paavo Havaksen tie 3, 90014, Oulu, Finland
Corresponding Author	Name: Paul V Debes; Address: Organismal & Evolutionary Biology Research Program / Institute of Biotechnology, 00014, University of Helsinki, Finland; Phone number: +358 2 9415 9537; Email: paul.debes@dal.ca; ORCID: https://orcid.org/0000-0003-4491-9564
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Abstract

Sexual maturation is a pivotal life-history trait that balances the probabilities between mortality and reproduction. Environmental vs. genetic contributions to maturation component traits, such as somatic growth and body condition, remain uncertain because of difficulties in determining causality. In Atlantic salmon, maturation timing associates with a large-effect locus around *vgll3*, which also links with growth, condition, and maturation in mammals. We investigate environmental vs. genetic contributions to maturation and its component traits by combining controlled breeding with common-garden experimentation in two temperatures. We test whether *vgll3* associates with first-year maturation of male salmon and, to avoid reverse causality, whether *vgll3* effects express via growth or condition in the males' non-maturing female relatives. Across 41 families, 4% of males matured in the cold vs. 39% in the warm environment. Maturation rate differed 3.3- to 4.6-fold between *vgll3* genotypes, which also explained around 30% of maturation heritability. Female condition differed up to 2% between *vgll3* genotypes, which also explained 6-17% of condition heritability. Non-significant *vgll3* effects on female length were antagonistic to those for condition but of equal proportional size. When accounting for *vgll3* effects, positive genetic correlations between male maturation and female growth increased, whereas those between male maturation and condition decreased, supporting an antagonistic effect of *vgll3* on growth and condition. The results indicate that large *vgll3* effects on maturation are mediated via large condition effects and suggest *vgll3* as a candidate locus for controlling the resource allocation trade-off between somatic growth and body condition.

Significance Statement

Identifying traits that affect sexual maturation timing and quantifying their relative environmental and genetic importance is a major goal in evolutionary research because of their strong links to fitness. However, cause and effect between maturation timing and such traits often remain unknown. We conducted a common-garden quantitative genetic study using Atlantic salmon to test for genetic contributions of body length and body condition expressed in immature females on maturation of their male relatives. We show that the detection of genetic associations between maturation and length or condition depends on both genetic and environmental factors and suggest that the genetic association with condition chiefly underlies a candidate gene with large effect on maturation that is mediated via variation in body condition.

Maturation timing is a central life-history trait that contributes to maximizing individual survival and reproductive success and, thereby, per *capita* population growth rate (1-3). Maturation timing is assumed to be genetically and environmentally controlled via factors including growth or size and body condition (1-5), which may signal current energy status (6, 7). However, disentangling cause and effect is a major challenge in studies on maturation and associated traits (8-10). For example, as opposed to growth inducing maturation, ongoing maturation can temporally increase growth, as is the case for the human puberty growth spurt. Likewise, the maturation process can lower growth and condition by competing with somatic growth and depleting reserves, respectively, or affect both via appetite (4, 5, 11). Related to this problem, fundamental evolutionary knowledge on presence and relative importance of genetic vs. environmental contributions to maturation timing and their link to maturation component traits, such as growth or condition, remains limited (12-15).

Contrary to the assumption that life-history traits are comprised of several underlying component traits, each of which are coded by many loci with small effect on phenotypic variation (1), maturation timing in Atlantic salmon (*Salmo salar*) associates with a locus explaining a large proportion of phenotypic variation (33-39%; 16, 17). This finding not only bears implications on evolutionary predictions (1, 18) but also offers outstanding opportunities for understanding the role of age-specific body size and condition and in quantifying the relative importance of their genetic vs. environmental contributions to maturation timing. The large-effect locus positions close to a transcriptional co-factor gene (*vgll3*), hypothesized as a strong candidate gene (16, 17). Known functions of Vgll3, such as inhibition of adipocyte differentiation in cell lines in favor of somatic growth processes and its negative transcriptional correlation with body mass and fat content in mice (19), suggest a mechanistic link to maturation via control of resource allocation as energy reserves vs. somatic growth. Genetic markers around *vgll3* have also been associated with salmon length (16), human maturation and growth (10), body condition (20), or condition change during maturation (21), fostering the idea that the link between *vgll3* and maturation, somatic growth, and condition underlies a common functional phenotype. However, a comprehensive joint assessment of *vgll3* effects on maturation, growth, and condition, which requires that the latter two traits are estimated free of maturation-induced changes, is still missing.

In Atlantic salmon, sexual maturation studies have a long history (5, 22, 23) and this species offers features allowing for a joint assessment of maturation, growth, and condition, thus enabling independent assessment of their links with *vgll3*. Specifically, readily available pedigreed hatchery populations allow for planned breeding of highly fecund individuals. Many offspring with tightly connected pedigrees combined with common garden experimentation followed by quantitative genetic analyses enable i) dissection of genetic from environmental effects, ii) estimation of genetic correlations between environments or iii) estimation of genetic, environmental, and phenotypic correlations between different traits. Perhaps the biggest advantage however, is the observation that Atlantic salmon males, but usually not females, can mature during their first year (23). This provides opportunities to estimate environmental and genetic contributions to maturation in males and those to growth and condition in non-maturing females. By comparing the latter

to those of their male relatives (of which some may show maturation-affected traits), and genetically relating male maturation to female traits, it is possible to infer the presence and relative importance of genetic vs. environmental components of maturation timing and its maturation-unbiased component traits.

Here, we implemented a quantitative genetic breeding design for 42 pedigreed Atlantic salmon parents with known homozygous *vgll3* genotypes and created >5,000 offspring males and females from 41 families with all *vgll3* genotypes (**Fig. S1**). Longitudinal study of these families in a common-garden environment enabled general estimates of relative genetic vs. environmental contributions for maturation timing of first-year Atlantic salmon and its component traits in conjunction with an assessment of specific *vgll3* effects on maturation, growth, and condition. Further, we evaluated whether a known association of the *vgll3* locus with maturation (co)expresses via growth, condition, or both, by testing whether male maturation probability correlates genetically with maturation-unbiased female somatic growth or body condition, or with both, and by quantifying the *vgll3* contribution to the genetic between trait correlation. By replicating all families in two environments with a life-time 2°C water-temperature difference across a seasonal temperature curve (a likely global-warming scenario (24); **Fig. S2**), we were also able to assess environmental influences of wide relevance on our estimates.

Results and Discussion

We used a multivariate generalized animal model to test for associations between *vgll3* and each of the five investigated traits of male maturation probability (maturation, MAT), sex-specific somatic growth (length, LEN), and sex-specific body condition (condition, CON). To avoid reverse causality (i.e., inferring condition- or length-mediated *vgll3* effects on maturation that are truly maturation-mediated *vgll3* effects on somatic growth or condition) we focused on *vgll3* effects on maturation in males and length and condition in females (of which none matured). To allow for wider interpretation and relevance of our results, we report maturation results, consistent with a genetic threshold model, on the probit or observational probability scale, which are relevant for breeding-value based or phenotypic selection, respectively (25). Likewise, we report results for length and condition on the proportional scale, which is relevant to growth processes at the individual level, or on the (phenotypic standard deviation, psd) standardized effects size scale, which is relevant among individuals at the population level.

When fully accounting for experimental randomization and relatedness, we detected strong *vgll3* effects on maturation probability and condition, but not on length, in both temperature environments, whereby the *vgll3*E* allele associated with both higher maturation and higher condition (**Fig. 1**). These results provide the first experimental confirmation that the allele associated with earlier maturation following marine migration (16, 17, 26) has consistent effects in males maturing in freshwater, and that the same allele also associates with a higher condition. All focal trait means were much higher in the warm than in the cold environment (**Fig. 1;** posterior temperature contrasts, 95% credible interval [95% CI]: probit scale, MAT_{Warm} - MAT_{Cold} = 2.0, 1.5-2.6; psd scale, female LEN_{Warm} - LEN_{Cold}= 1.5, 1.3-1.7; female CON_{Warm} - CON_{Cold}= 0.92, 0.57-1.29). Despite these strong environmental effects on trait means, *vgll3* effect estimates did not differ between environments for any trait (**Fig. S3**).

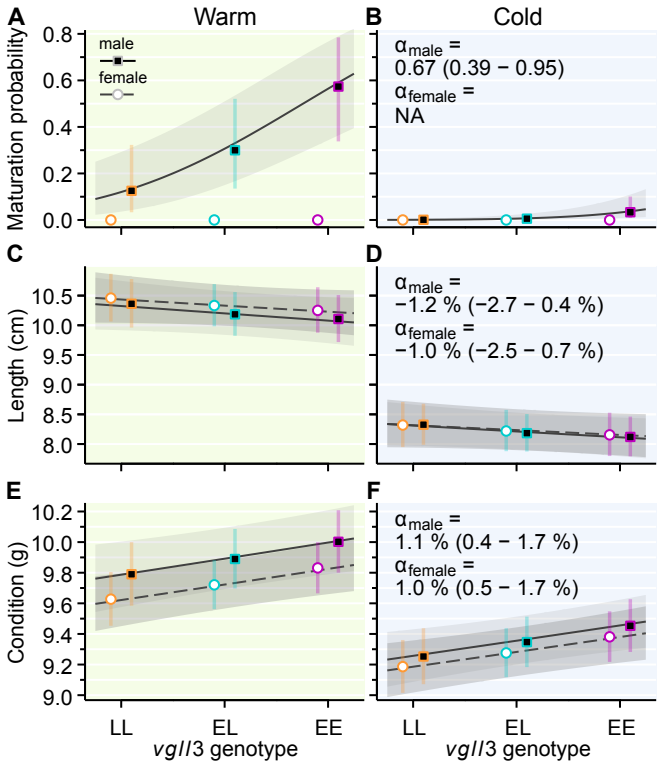

Fig. 1. ***Vgll3*** **genotype mean estimates and additive** ***vgll3*** **effect estimates for the sex-specific responses of Atlantic salmon maturation, length, and condition.** The panels show back-transformed *vgll3* genotype-specific mean estimates with 95% credible intervals for sex-specific maturation probability (**A, B,** no female maturation occurred), body length (**C, D**), and body condition (standardized mass for a geometric mean-sized fish of 9.1 cm, **E, F**) from a common multivariate generalized animal model ($N_{families}$ = 41, N_{male} = 2,534, N_{female} = 2,611). Estimates are shown for either a warm or cold temperature environment. Sex-specific regression lines with 95% credible bands show additive *vgll3* effects estimates (α) that are either estimated across both temperature environments (maturation, length, female condition) or that are estimated for each environment (male condition). Mean α estimates with 95% credible intervals are also shown. Mean *vgll3* genotype estimates and *vgll3* α estimates originate from similar multivariate models differing in whether *vgll3* effects were specified either as factorial or continuous terms, respectively.

We also formally tested jointly for additive (α) and dominant (δ) effects for all traits, because sex-specific dominance (i.e., *vgll3*-by-sex epistasis) has been reported for sea age at maturity in one of two previous studies (16, but see 26). We did not detect dominance for any trait (**Fig. S4**), which verified a condition for meaningfully associating *vgll3* effects between male and female traits that would not be given under sex-specific dominance. After removing all dominance effects from the model, we estimated common additive effects for all traits across temperature environments (**Fig. 1 B, D, F**). For female length, we estimated a non-significant but negative additive *vgll3**E allele effect on the psd scale of α = -0.06 (95% CI = -0.16-0.05). This effect translates to a non-significant 1% lower body length per *vgll3**E allele (**Fig 1 D**). For female condition, we estimated an additive *vgll3**E allele effect on the psd scale of α = 0.20 (95% CI = 0.09-0.33). This among-individual additive *vgll3* effect on condition is much larger than that estimated for length. However, it translates to the same proportional effect as estimated for length, but in the opposite direction. Specifically, a non-significant 1% body length decrease per *vgll3**E allele is accompanied by a significant 1% body mass increase (**Fig 1 F**). Although the *vgll3* effect on length is non-significant and of little, if any,

importance at the population level (but may still represent a within-individual trade off, discussed below), the same effect size of 1% body mass (condition) increase per *vgll3**E allele is very likely relevant for maturation. Specifically, male salmon gonad development is dependent on body reserves, such as fat (4, 5), and gonads constitute 5-9% of body mass at spawning (27, 28). Thus, condition variation explained by *vgll3* genotype may finance up to 40% of the required extra mass (ignoring unknown reserve-to-gonad mass conversion factors). These results provide the first indication that the large effect of *vgll3* on maturation is, at least partly, mediated by a large effect of *vgll3* on condition. We strengthen this further by providing additional evidence below where we quantify *vgll3* effects on trait-specific heritabilities and between-trait genetic correlations.

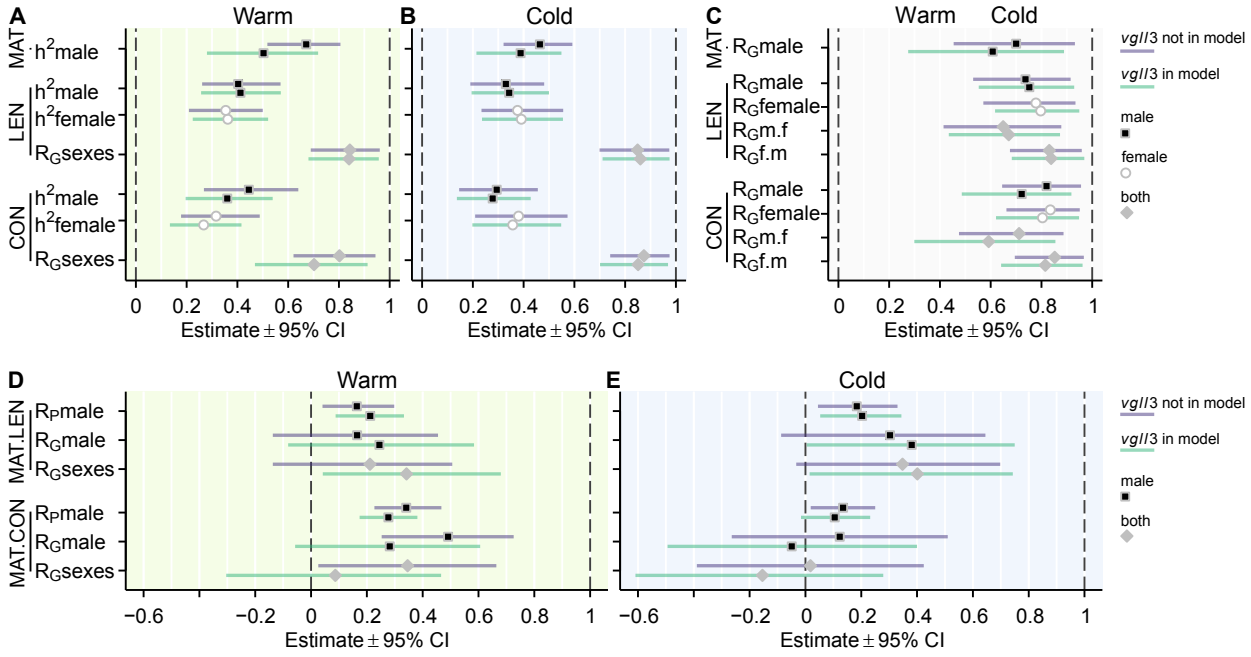

Fig. 2. Heritability and genetic correlation estimates from a multivariate generalized animal mixed model, for the traits of Atlantic salmon male maturation (MAT), sex-specific length (LEN), and sex-specific condition (CON). The top row shows, for each trait, sex-specific heritabilities (h^2) and the sex-specific or between-sex genetic correlations (R_G), estimated either specific for each temperature environment (**A, B**) or between temperature environments (**C**; genetic between-environment correlations within and, reciprocally, between sexes). The lower row shows phenotypic (R_P) and genetic correlations (R_G) between maturation and length (MAT.LEN) or maturation and condition (MAT.CON) within sexes and between sexes (only for R_G) in the warm (**D**) and cold (**E**) environments. All estimates are depicted with 95% credible intervals. All estimates were obtained by two similar multivariate models differing in whether additive *vgll3* effects were included or excluded as mean effects as indicated in the legends.

The multivariate model also enabled us to estimate environment- and trait-specific heritabilities (h^2) and trait-specific genetic correlations (R_G) between temperature environments (both contribute to genotype-by-environment interactions (29)) and also to estimate how much *vgll3* contributes to either. The heritability of a trait quantifies the proportion of phenotypic variance explained by the sum of all underlying additive genetic effects and predicts the response to selection. The genetic between-environment correlation quantifies the genotype re-ranking between environments and predicts, in conjunction with heritability estimates, the response to selection across environments (30). Fitting each model with and without mean-

effect specified additive *vgll3* effects, enabled us to quantify the relative genetic and phenotypic contributions of additive genetic *vgll3* effects and thus the trait-specific evolutionary importance of *vgll3* within and across environments.

Remarkably, male maturation heritability (h^2MAT) on the probit scale was three times larger in the warm environment than in the 2 °C colder environment (**Fig. 2 A, B;** posterior temperature contrast, 95% CI; h^2MAT_{Warm} - h^2MAT_{Cold} = 0.32, 0.21-0.41). Translated to the observed scale, maturation heritability was four times higher in the warm environment (posterior mean, 95% CI; h^2MAT_{Warm} = 0.41, 0.32-0.50; h^2MAT_{Cold} = 0.10, 0.04-0.16; posterior temperature contrast, 95% CI; h^2MAT_{Warm} - h^2MAT_{Cold} = 0.28, 0.17-0.38;). Further, the between-temperature genetic correlation estimate (R_GMAT) for maturation was < 0.8 (posterior mean, 95% CI; $R_GMAT_{Warm.Cold}$ = 0.70, 0.45-0.93), which is somewhat lower than most previous genotype-by-temperature environment estimates for maturation of related species (29). Importantly, R_G < 0.8 indicates breeding value re-ranking between environments with a mere 2°C temperature difference and thus is of relevance to natural and aquaculture settings (29, 31). This is of particular relevance, in combination with the pronounced differences in maturation heritability (30), under a 2 °C global-warming scenario (24). For maturation, additive *vgll3* effects explained 29.3 and 31.2% of the observed-scale heritability and 12.0 and 10.0% of the observed-scale phenotypic variance in the warm and cold environments, respectively. On the probit scale, additive *vgll3* effects explained 29.9 and 31.9% of heritability and 13.8 and 2.9% of phenotypic variance, respectively. Additive *vgll3* effects also explained 11.4% of the between-environment genetic correlation for maturation (**Fig. 2 C**), which supports an interpretation that the abovementioned non-significant *vgll3*-by-temperature interaction effects imply environmentally stable *vgll3* effects relative to other additive genetic effects on maturation.

Female length heritability was, in contrast to that for maturation probability, similar between environments (posterior mean, 95% CI; h^2LEN_{Warm} - h^2LEN_{Cold} = -0.03, -0.19-0.13) and the between-environment genetic correlation of $R_GLEN_{Warm.Cold}$ = 0.78 (0.58-0.94) indicated only minor genotype re-ranking between environments (**Fig. 2 A, B, C**). This between-environment genetic correlation contrasts with much lower estimates in other fish species (29). Accounting for *vgll3* effects, unexpectedly, increased female length heritability, as opposed to lowering it as observed for maturation heritability, though by only 1.7 and 6.2% in the warm and cold, respectively (**Fig 2 A, B, C).** When we investigated underlying length variance components between models, we found that including *vgll3* effects re-allocated variance from the residuals to the additive genetic component, which is not expected if *vgll3* comprises additive genetic effects on length.

Female condition heritability was, like that for female length and in contrast to that for maturation probability, similar between environments (h^2CON_{Warm} - h^2CON_{Cold} = -0.09, -0.30-0.08) and the between-environment genetic correlation of $R_GCON_{Warm.Cold}$ = 0.84 (95% CI = 0.71-0.96) indicated very little genotype re-ranking between environments (**Fig. 2 A, B, C**). Additive *vgll3* effects explained 16.2 and 6.5% of the condition heritability and 5.2 and 2.5% of the phenotypic variance in the warm and cold environments, respectively. And, *vgll3* effects also explained 4.4% of the between-environment genetic correlation for female condition

(**Fig. 2 C**), which is noticeable but less than what *vgll3* explained for maturation of the same parameter. Thus, these results support, like for maturation probability, the presence of environmentally stable *vgll3* effects, but in the presence of a relatively more environmentally stable genetic background for condition than for maturation.

By quantifying the proportion of the additive *vgll3* effect contribution to trait-specific heritability, we were unable to support a hypothesized *vgll3* association for male maturation via female length but support an association for male maturation via female condition. Quantitative contribution estimates of the genetic marker to the heritability were sufficiently large to consider *vgll3* a large-effect locus for both maturation and condition. Nonetheless, *vgll3* contributions to the phenotypic maturation-timing variance were smaller than previous estimates at a later life stage (16, 17, 26), which could have several biological, methodological, or statistical reasons, discussion of which goes beyond the scope of this manuscript. Furthermore, by having observed the same direction for *vgll3* effects between male maturation and female condition, we had obtained the first indication that *vgll3*-induced variation for male maturation and female condition may underlie a common *vgll3*-related molecular process. We wanted to further foster this idea by quantifying the *vgll3* contribution to the genetic correlation between male maturation and female length (MAT.LEN) and between male maturation and female condition (MAT.CON). To do so, we had borrowed quantitative genetic methodology from animal breeding (an extension of methods reviewed in 32), whose implementation through the covariance structure of the multivariate animal model was possible by our study design, and estimated the between-sex genetic correlation between different traits.

We did not detect genetic correlations between male maturation and female (or even male) length, although estimates in both environments were positive and the relationship-controlled phenotypic correlation estimate (R_P) (33) between male maturation and male length was significant in both environments (**Fig 2 D, E**). All correlation estimates (genetic and phenotypic) between male maturation and female or male length increased after accounting for *vgll3* effects (**Fig 2 D, E**). Thus, we were able to further exclude length as a mediator of *vgll3* effects on maturation, which requires inferring a positive genetic covariance among *vgll3* effects between traits, but instead found a statistical behavior that supports a negative genetic covariance among *vgll3* effects between maturation and length.

Between male maturation and female (and male) condition, we, however, detected a positive genetic correlation, although only in the warm environment (we discuss this environmental difference below). After accounting for *vgll3* effects, all genetic correlations between male maturation and female or male condition decreased, as is expected under scenarios of either the presence of pleiotropic *vgll3* effects on condition and maturation, or when one trait mediates the genetic-marker associated variation of the other. The decreasing effect was strongest for the genetic correlation between male maturation and female condition, which even rendered non-significant when accounting for *vgll3* effects. Ignoring a *vgll3* pleiotropy scenario, this latter result suggests a positive genetic covariance among *vgll3* effects between male maturation and female condition, which may even be dominated by *vgll3* effects and, thereby, suggests a *vgll3* effect

mediation on maturation via condition and not *vice versa* (because maturation variation cannot account for condition variation in immature females).

Although many effects for length were statistically non-significant, we cannot entirely rule out small *vgll3* effects on length. Generally, growth or length and maturation initiation are assumed to associate positively (4, 5). By inferring a negative covariance among *vgll3* effects between maturation and length we were able to rule out length mediating *vgll3* effects on maturation. However, the inferred negative genetic covariance among *vgll3* effects between maturation and length support a scenario for a *vgll3*-governed resource allocation trade-off. Such a trade-off was also, albeit weakly, indicated by the non-significant 1% length decrease per *vgll3**E allele that was accompanied by a significant 1% condition increase. This result is typical in life-history research for traits that represent within-individual resource allocation trade-offs in the presence of much larger among-individual variation for resource acquisition relative to the within individual resource-allocation conflict (1). The idea of a *vgll3*-mediated resource allocation trade-off also makes biological sense. This is not only because resource allocation theory predicts that energy allocated to condition cannot concurrently be allocated to growth, but also due to the suggested role of Vgll3 in controlling mesenchymal cell fate into either adipocytes (thus increasing body condition) or bone and cartilage lineages (thus increasing somatic growth) (19). However, a final conclusion for our tentative results requires additional research.

A remaining question is why we detected a significant genetic correlation between male maturation and female condition in the warm, but not the cold, environment. We suspect that the higher maturation rate and higher maturation heritability in the warm provided sufficient information to detect the genetic correlation (31). In contrast, many males in the cold environment with high breeding values for condition did not mature because of the much lower average condition in the cold or due to other lower temperature-related causes, such as not exceeding growth or size thresholds needed for maturation (4, 5), masking an otherwise positive genetic correlation.

Extending these thoughts, we detected environmental effects on condition that were consistent with the notion that both condition and maturation covary positively with temperature. Specifically, relative to the cold environment and when translated to the proportional scale we detected a 4.6% (95% CI = 2.9-6.4%) higher female condition in the warm environment, where we had also detected a much higher maturation probability (**Fig 1 A, B, E, F**). Thus, the environmental effect of a global-warming-relevant 2°C temperature difference (24) on condition was more than twice the maximum genetic *vgll3* effect (EE vs. LL: 2%) and may have contributed considerably to the large environmental effect on maturation probability. Similar positive relationships between body condition and water temperature have previously been observed (34, 35). We suspect that temperature effects on maturation via condition explain, at least partly, unexpectedly high maturation rates in heated salmon rearing facilities (36) and why effects on size and maturation timing vary between growth acceleration through increase in feed vs. temperature (37, 38). Important for consistency of natural and human selection success across temperatures (30), the large average condition temperature difference observed here was evident in the presence of both high genetic correlations (> 0.8

for both sexes) and similar condition heritabilities between temperatures (**Fig 2 G, H, I**). Furthermore, *vgll3* effect estimates on both female condition and on male maturation did not differ between temperature environments, and *vgll3* effects contributed to the between-environment genetic correlations for both traits. Thus, the environmental stability of *vgll3* effects on condition contributes to the environmental stability of the remaining additive genetic effects on condition.

These new results suggest - together with the previous results on maturation (16, 17, 26) - the presence of environmentally stable *vgll3* effects on both condition and maturation. A large share of *vgll3* effects for the positive genetic correlation between maturation and condition predicts rapid evolutionary co-responses to selection for either trait, but also predicts that their genetic correlation is sensitive to *vgll3* allele frequencies. The sex-specific results, together with previous biological knowledge on their causal association (4, 5), indicate that large *vgll3* effects on maturation are likely mediated via large condition effects and suggest *vgll3* as a candidate locus for controlling the resource allocation trade-off between somatic growth and body condition.

Materials and Methods

Fish population, breeding and experimental design, data collection

The experimental cohort was parented by pedigreed hatchery fish maintained by the Natural Resources Institute Finland (Laukaa, Finland). The hatchery-stock ancestors originated from the River Neva, Russia, which drains into the Baltic Sea. In November 2017, we crossed 48 parents with known *vgll3* genotype as 12 2x2 factorials of unrelated *vgll3* homozygous individuals; each factorial yielded four reciprocal *vgll3* offspring genotypes (EE, EL, LE, LL; details on realized design and resulting pedigree in **Fig. S1**). We reared the experimental cohort in a recirculation system controlled for water temperature, oxygen, dissolved nitrate components, and natural light cycle, which affect growth or sexual maturation timing (4, 5). We split each family by randomizing equal number of individuals into two egg-incubator replicates (families separated; kept in darkness) and, at first feeding and after pooling incubator replicates, into eight similar tank replicates for each of two water temperatures (totaling four incubators and 16 tanks). Water temperatures followed a seasonal cycle with a 2°C difference, referred to as “warm” or “cold” (warm, range = 6.3-17.7 °C; cold, range = 4.1-16.0 °C; **Fig. S2**). Fish were fed *ad libitum* using a commercial salmon diet starting 2018-03-09 (warm) or 2018-04-27 (cold) (**Fig. S2)**. Once fish size allowed passive integrated transponder tagging (warm: August 2019; cold: September 2019) to enable re-identification, we anesthetized (using methanesulfonate), fin clipped, and tagged individuals; fin clips allowed for genotyping individuals to assign family, determine molecular sex, and confirm *vgll3* genotype. Starting at tagging, we followed trajectories for fork length (± 1 mm) and wet mass (± 0.01 g) in 3-6-week measurement intervals until final spawning time (December 2018) when we determined maturity status by checking for extruding milt by gently pressing the abdomen and confirmed sex and maturity status in 84% of the fish by culling and dissection (N = 4,313). Once individual identification was possible, we applied a feed-restriction treatment (either *ad libitum* feeding for seven days per week or *ad libitum* feeding for two days per week with no feeding for two or three days between feedings) that was crossed with the temperature treatment

for a five-week period (September 2018), but we did not detect any feed-restriction effect on maturation (**Fig. S5**). Because length and condition data included in models originated from the earliest common time point, before the feed restriction, we omitted the feed restriction term from further analyses. Animal experimentation was conducted according to license ESAVI-2778-2018.

Genotypes and molecular sex of both parents and the experimental cohort were determined using a multiplex-PCR for 177 single nucleotide polymorphisms (SNPs) of a previously described SNP panel (39), followed by Ion Torrent (988 potential parents) or Illumina sequencing (used parents, experimental offspring cohort). Using a subset of 141 unlinked, polymorphic SNPs, we reconstructed the parents of potential parents (the experimental cohort's grandparents) with maximum likelihood (40) and assigned the experimental individuals to their parents with a likelihood approach (41). Merged information about reconstructed grandparents and assigned parents of the experimental cohort yielded a three-generation pedigree (**Fig. S1 B**) on which we based the relationship matrix utilized in animal-model analyses (42).

Data analysis

We fitted a series of uni- and multivariate general and generalized linear animal models to data for maturation binaries, assessed towards the end of spawning time in December, and length and condition records, assessed four months before spawning time in late summer. We defined condition as deviation from the (temperature-specific) slope of logarithmic mass on logarithmic length - a correlate of salmon parr lipid content (43). Length and condition data were first log-transformed, then mean-centered and variance scaled to estimate biologically meaningful proportional and phenotypic variance-standardized effects.

To determine the multivariate model structure and allow for comparisons between uni- or bivariate model estimates with multivariate model estimates (**Fig S7**), we first fit univariate models for the binary response of male maturation and bivariate models for the continuous responses of sex-specific length or condition. We estimated means and (co)variances for models with the binary maturation response (including the multivariate model) using Bayesian Markov Chain Monte Carlo simulations (44) with the R-package MCMCglmm (45). For the continuous responses, we initially fitted bivariate models under REML (44) using ASReml (46). We then fit the multivariate model corresponding to the chosen univariate (maturation) or bivariate models (sex-specific length or condition), and by adding the required between-trait covariances for the additive genetic effects (2,599 [males] or 5,209 [both sexes] relationship-matrix-predicted breeding values (Henderson 1973) *alias* animals), common environmental effects (16 tanks), maternal effects (21 dams), and individual environmental effects including measurement error and non-additive genetic effects (2534 [males] or 5145 [both sexes] residuals). We fitted the models with probit-link function and residual variance fixed to one for maturation, corresponding to genetic threshold models (47), and with identity link function for length and condition. We did not detect maternal effects on any trait (**Fig. S6, Table S1, Table S2**; we removed dam effects as a consequence) and no common environmental effects on maturation (**Fig. S6;** we still kept the experimental tank effects), but on length and condition, which contributed up to 6 and 15% to the total phenotypic variance, respectively (**Fig. S7**). We started model selection with mean-effect interactions and removed non-significant effects (**Fig. S3, S5**). Final models followed the general equation

(per trait), with colon indicating term interaction and variance terms in italic: y ~ Intercept + Temperature + Vgll3 + *Temperature:Tank* + *Temperature:Animal* + *Temperature:Residual*. The Vgll3 model term refers to either three genotypes (reciprocal heterozygote differences were absent; posterior EL-LE contrast, 95% CI: MAT_{EL}-MAT_{LE} = 0.00, -0.47-0.49), or to the additive allelic effect (we fitted both). Covariance matrices across temperatures for initially fit univariate models were diagonal for tank and residual effects and unstructured for dam and animal effects. The latter allows estimating dam- and additive genotype-by-environment effects (30). We used (co)variance priors following a χ_1^2 distribution in Bayesian univariate models (44) or priors that resulted in flat priors for heritabilities and correlations in multivariate models. We based estimates on 5,000 or 1,000 retained iterations after 50,000 burnin iterations and sampling every 1,250 and 2,500 or 3,000 iterations (uni- and multivariate models, respectively). We confirmed model convergence by trace plot inspection and ensuring lag-two autocorrelation < 0.1 for mean and (co)variance estimates. For the multivariate models (five response traits: sex-specific length and condition, male maturation) we expanded the covariance matrices to temperature-specific block-diagonals for *Trait:Temperature:Tank* (two 5x5 blocks), a full *Trait:Temperature:Animal* genetic covariance matrix (10x10), and temperature- and sex-specific block-diagonals for *Trait:Temperature:Residual* (two 2x2 female and two 3x3 male blocks).

We tested and estimated additive and dominant *vgll3* effects by replacing the factorial Vgll3 term by appropriate covariates (α: -1, 0, 1; δ = 0, 1, 0). To obtain observed-scale maturation probability parameter estimates, we integrated over marginal model predictions and used methodology implemented in the R-package QGglmm (25), applied to each retained MCMC iteration to estimate credible intervals. We calculated genetic, environmental, and phenotypic correlations following Searle (33). All necessary data and an R-script mirroring the final uni- and multivariate modeling have been deposited in the DRYAD data repository (available during review, doi after acceptance).

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Author contributions

P.V.D. and C.R.P. designed research; P.V.D., N.P., A.R., O.O., J.E.M.-V., N.P., T.A., and C.R.P. performed research; J.E. contributed salmon gametes; P.V.D. analyzed data; P.V.D. and C.R.P. wrote the paper.

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