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Source of additive genetic variance of important traits

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Abstract

Fisher's fundamental theorem of natural selection predicts no additive variance of fitness in a natural population. Consistently, observations in a variety of wild populations show virtually no narrow-sense heritability (h^2) for traits closely related to fitness. However, counterexamples are also reported, calling for a deeper understanding on the evolution of additive variance. In this study we propose adaptive divergence followed by population mixture as a source of additive variance of fitness-related traits. We experimentally tested the proposal by examining a panel of ~1,000 yeast segregants produced by a hybrid of two yeast strains subject to adaptive divergence. We measured over 400 yeast cell morphological traits and found a strong positive correlation between their h^2 and their relatedness to fitness. This pattern, being a counterexample of the prediction of Fisher's theorem, well supports our proposal. Because adaptive divergence followed by population mixture could happen constantly, particularly in some species including humans and domesticated animals or crops, the proposal provides a framework for reconciling the availability of abundant additive variances of important traits with Fisher's fundamental theorem of natural selection.

Introduction

A basic issue in genetics and evolution is understanding the relationship of natural selection and fitness (Orr 2009; Hendry, et al. 2018). The Fisher's fundamental theorem of natural selection states as: "The rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time" (Crow 2002). It predicts that there should be no additive variance (or narrow-sense heritability, h^2) of fitness in an equilibrated population, because natural selection will fix alleles with the highest fitness quickly (Mousseau and Roff 1987; Merila and Sheldon 1999a). An extended prediction of the theorem is that traits associated with fitness should have small h^2 than traits unassociated with fitness (Kruuk, et al. 2000). The underlying

logic is response to natural selection on fitness causes evolutionary response at other traits (Orr 2009). Numerous studies have echoed this prediction in different wild populations (Merila and Sheldon 1999b; Kruuk, et al. 2000; Merila and Sheldon 2000; Stirling, et al. 2002; Teplitsky, et al. 2009; Wheelwright, et al. 2014; Sztepanacz, et al. 2017). For female red deer (Cervus elaphus), the values of h^2 for life history traits such as total fitness, adult breeding success, and longevity were zero (Kruuk, et al. 2000). Morphologic traits have higher h^2 than life history traits in red deer, collared flycatcher (*Ficedula albicollis*), Savannah sparrows (Passerculus sandwichensis), red-billed gull (*Larus novaehollandiae*), et al (Kruuk, et al. 2000; Merila and Sheldon 2000; Stirling, et al. 2002; Teplitsky, et al. 2009; Wheelwright, et al. 2014). However, additive variances have been detected in many other empirical observations (Price and Schluter 1991; Pettay, et al. 2005; Teplitsky, et al. 2009; Kosova, et al. 2010; Zhang 2012). For example, h^2 ranged from 0.175 to 0.563 for the same traits in female preindustrial humans (Pettay, et al. 2005). A well acceptable explanation of maintaining additive genetic variance is: the different estimations of fitness we usually used are different fitness components actually, and there are tradeoffs between fitness components which suffer balancing selection under the influences of environment, sex, and so on (McFarlane, et al. 2014; Hendry, et al. 2018). However, the source of additive variances of fitness is not clear enough because various explanations like above are usually context-dependent.

Ho et al. found faster evolution of more important morphological traits within and between species, supporting the adaptive hypothesis of phenotypic evolution (Ho, et al. 2017). The adaptive divergence followed by population mixture may provide a clue to understand why additive variance of fitness traits are observed in some wild populations. A hybrid population course the process of separation, hybridization, and adaptation (Fig. 1). For each parent, they evolve independently since their divergence. When the two populations mix again, the states of different loci in the hybrid offspring need to adapt the new genetic background rapidly. The null model of the relationship between heritability and traits could be one of the three types: non-correlation, positive, or negative. For specific loci, there are numerous combinations of them, which could be summarized to non-additive or additive ways. To verify which model is suitable for hybrid populations, we have measured ~400 morphological traits and fitness for each strain in a segregant panel. This panel consists of ~1,000 prototrophic haploid segregants from a cross between two yeast strains which were generated and genotyped in a recent study (Bloom, et al. 2013). The parental strains of the segregant panel are two intra-species (BY parent and RM parent) with 0.5% differences at the sequence level. These data together make it possible to investigate Fisher's fundamental theorem in a hybrid population.

Results

Measuring ~400 morphological traits of each segregant

Yeast morphological traits were carried out by Ohya et al to quantify the cell and nuclear DNA morphology by analyzing images of staining cell membrane and nucleus

(Ohya, et al. 2005). These traits are related to the characters of mother cell and/or bud, such as area, distance, localization, angle, ratio and so on (Fig. 2A). We verified the segregant panel and removed the strains which were mismatched with the reference genotypes (Methods). The morphological traits of each strain were measured following Ohya's protocol with some modifications (Methods). Two replications of each segregant were measured independently, and segregant A11_01 and A11_96 were measured in every experiment as a technical control to estimate the operating bias in culturing, staining, and imaging. We focused on the strains whose cell number for calculating traits were more than 80. There were total 734 segregants each with 405 morphological traits derived, in which 73.3% (538/734) had at least two replications.

Over 99.5% of traits were calculated from >100 cells (Fig. S1). Pairwise correlations of 405 morphological traits between different replications of A11_01 (26 replications) and A11_96 (28 replications) suggested that the experimental repeatability was very good (Fig. 2B & Fig. S1). Ninety-six percentages of pairwise correlations of A11_01 were larger than 0.9, and the proportion for A11_96 was nearly 100%. The value of CV of each trait in A11_01 was highly correlated to A11_96 (Pearson's R = 0.76, $P < 2.2\times10^{-16}$; Fig. 2C), showing the reliability of morphological data set. Therefore, we conducted a reliable data set of morphological traits for the segregant panel (Table S1). The growth rates of each strain in the same culture were also measured (Methods, Table S2). Combined with genotype information, it provided an opportunity to study heritability and traits with different importance but equivalent property in a large scale simultaneously.

Estimating heritability of ~400 morphological traits

For each of 405 traits, we first estimated broad-sense heritability (H^2) from repeatability of traits and narrow-sense heritability (h^2) from mean value of each trait according to the approach developed by Bloom et al (Bloom, et al. 2013) (Table S3). As the same circumstance to Bloom's study, there is no dominance effects and gene-environment interactions because segregants are haploid and measured under identical conditions, respectively. Thus H^2 of each trait includes additive effects and gene-gene interactions, and h^2 includes only additive effects. As shown in Fig. 2D, though calculated by the consistent rules of the same staining image, morphological traits have a large range of heritability. Among 405 morphological traits, H^2 ranges from 0.025 to 0.885, with a median of 0.473. Narrow-sense heritability ranges from 0.000 to 0.608, with a median of 0.183. It also showed that the additive component (h^2) contributed to a large proportion of heritability. The patterns held by traits in different categories divided by staining dye or cell cycle stage (Fig. S2 & Fig. S3), as well as exemplary traits that were less related with each other (Fig. S4).

We also estimated coefficients of additive genetic (CV_A) and residual (CV_R) by $CV_A = \sqrt{V_A}/\bar{X}$ and $CV_R = \sqrt{V_R}/\bar{X}$, respectively, where V_A is the additive variance, V_R is the residual variance, and $\bar{X}$ is the mean of each trait. A strong positive correlation between CV_A and CV_R was exhibited in Fig. 2E (Pearson's R = 0.802, $P < 2.2\times10^{-16}$), but the correlations between h^2 and CV_A or between h^2 and CV_R were weak (Pearson's

R = 0.15, P = 0.002 for CV_A; Pearson's R = -0.28, P = 6.5×10^{-9} for CV_R; Fig. S5).

We then mapped quantitative trait loci (QTL) for each trait. A total of 2,317 QTLs for 391 traits were detected (Table S4). The number of QTL ranges from 1 to 16, with a median of 5 (Fig. S6). No QTL was found for 14 traits, which conformed to extremely low h^2 of these traits (median h^2: 0.006). The phenotypic variances explained by QTLs were almost equivalent to h^2 (Fig. 2F), which was consistent with the observations in a previous study (Bloom, et al. 2015). Over 75% QTLs explained less than 3% phenotypic variances respectively (Fig. S7), which indicated that the amount rather than the effect size of QTL determined the narrow-sense heritability for yeast. Taken together, absolute additive component may dominate the level of h^2.

Positive correlation between heritability and trait importance

Fitness reflects natural selection directly. The relatedness to fitness of each trait represents the trait importance. Across 405 morphological traits, we used correlation coefficient (Pearson's R) between growth rates and mean values of each trait for 734 segregants to quantify the relatedness to fitness, following the general definition in other studies (Orr 2009; Chen, et al. 2017; Hendry, et al. 2018). The relatedness to fitness of these traits varies from 0 to 0.303, with a median of 0.068 (Fig.3A). A strong positive instead of negative correlation was revealed between h^2 and relatedness to fitness among 405 traits (Pearson's R = 0.567, P < 2.2×10^{-16}). This result suggests that more important traits have more additive variances, which supports the positive model and contradictory to the pattern revealed in wild animals.

To confirm the relationship between h^2 and trait importance, we investigated another index of trait importance. As it has been suggested by Ho et al, traits with smaller CV are more important to organismal survival and reproduction, underlying important traits are environmentally robust (Ho and Zhang 2014). There were dozens of replications for segregants A11_01 and A11_96, so we could estimate the environmental robustness of each trait. We calculated mean value of CV of A11_01 and A11_96 for each trait, and excluded the traits with CV distance of two groups larger than 0.2 further for strict (methods, Fig. S8). Among the left 298 traits, a negative correlation existed between h^2 and mean CV (Pearson's R = -0.33, P = 4.35×10^{-9}; Fig. 3B). This result also points to that important traits have a relative high heritability. A trait set including 87 important traits and 87 unimportant traits was defined by considering both relatedness to fitness and mean CV (Methods). Important traits had higher CV_A, lower CV_R, and more QTLs than unimportant traits, and there was no difference of phenotypic variances explained by single QTL between two groups of traits (Fig. S9).

Discussion

Fisher's fundamental theorem of natural selection exhibits a different face in the yeast hybrid population. The admixture benefits in a hybrid population such as increased genetic variation or novel genotypes are concerned because they are importance to population fitness, though the acting way of selection is not clear enough

(Verhoeven, et al. 2011). The adaptive divergence followed by population mixture model provides a possible mechanism. In the re-equilibrated process after hybridization, natural selection needs to fix various alleles from distinct genetic backgrounds. More important traits would suffer stronger selection. For such a trait associated with a certain number of loci, the most convenient way of selection is summing the effects of each loci additively, which means that important traits have additive variances under such circumstance. Our results have demonstrated that point: more importance traits have more additive variances.

For wild populations, many factors such as different time span or definition for fitness and non-fitness traits, strong environmental noise, bring all kinds of uncertainty to the measurement of traits (Visscher, et al. 2008). It is no surprise that there are many inconsistent observations in different wild animals. For example, opinions vary as to the cause of lower heritability of fitness traits – the debate between lower additive variance (estimated by V_A or CV_A) and higher environmental variance (V_E) or residual variance (CV_R) (Merila and Sheldon 2000; Pettay, et al. 2005; Teplitsky, et al. 2009; McFarlane, et al. 2014; Wheelwright, et al. 2014; Sztepanacz, et al. 2017). Besides, the negative correlation between h^2 and relatedness to fitness even is absent in some cases. For a bighorn sheep population from Ram Mountain, the lowest heritability was for body mass at primiparity (0.02), but heritability of longevity and lifetime fecundity were 0.46 and 0.66, respectively (Reale and Festa-Bianchet 2000).

In contrast, the panel of segregants have advantages: firstly, as a general rule, growth rate which is easy to measure can be taken as fitness (Orr 2009); secondly, traits can be measured under uniform conditions, which is an effective way to control measurement errors; thirdly, no maternal effect needs to be considered; fourthly, because of random recombination and meiosis, genotypes in the panel are various, so the shared epistasis effects which is common in limited related individuals could be largely controlled. More details were revealed by comparative analysis of the data of segregants. For traits with the same level of trait importance (defined by threshold of relatedness to fitness: 0.1; corresponding threshold h^2: 0.226; Methods), the level of h^2 related to CV_R but not to CV_A (Fig. S10). But between traits different both in levels of h^2 and importance, both CV_A and CV_R showed significant differences (Fig. S10). These details provide a clue to explain why different wild populations draw different conclusions: it would make little sense to pay close attention to the absolute values of variance components of different traits when ignoring their comparability.

In conclusion, we have confirmed that additive variances exist in the adaptive process of a hybrid population. More important the trait is, larger heritability the trait has. We suggest the additive variances may be due to the strong selection in important traits in the adaptive divergence followed by population mixture, which provide a new insight to understand Fisher's fundamental theorem in unequilibrated populations.

Materials and Methods

Verify segregant panel. The segregant panel was kindly provided by Dr. L. Kruglyak. There were total 1,056 segregants in eleven 96-well plates. To verify the genotypes, twelve strains in each plate were randomly picked up and four loci (MATa, MATα, hphMX4, natMX4) were amplified by polymerase chain reaction (PCR) for these strains. By comparison the results with the genotypes provided by Dr. L. Kruglyak, we found that some percentage of strains in Plate 8 and 9 were mismatched, and there was no pattern to rescue the strains in a row or a line, which may be the result of contaminations. We then focused the strains in the left nine plates with right genotypes in the next experiments.

Measure morphological traits. The morphological traits of each strain were measured following Ohya's protocol with some modifications (Ohya, et al. 2005). Briefly, strains were grown in YPD medium (yeast extract/peptone/dextrose medium) to saturation phase at 25°C for two or three days, and then transferred to new cultures to exponential phase at 25°C for three or four hours. Cells were fixed with 3.7% formaldehyde solution. Cell walls were stained with FITC-ConA (fluorescein isothiocyanate-conjugated, concanavalin A, Sigma-Aldrich C7642). Cell nucleus were stained by hoechst-mix (Thermo Fisher, Hoechst 33342 Solution) instead of DAPI to enhance the specificity. We omitted the process of actin staining because the dye of actin (Rhodamine phalloidin) was not stable and couldn't support to image for a long time in the high-throughput automated image-processing. The stained cells were plated on microplates (Greiner 781091) with ~5.0×10^4 cells per well and taken images by IN Cell Analyzer 2200 (GE Healthcare) with 100× objective lens. There were two technical replications for each segregant, and segregants A11_01 and A11_96 were stained and imaged in every experiment as a technical control.

CalMorph software was used to analyze images to quantify yeast morphology, and 405 quantitative traits were derived. Values of all traits were listed in Table S1. Traits derived from cell wall or nucleus can be distinguished by the initial letter of traits, which "C" is related to cell wall, and "D" is related to nucleus. Traits in different stages can be distinguished by the letters after the connector line. "A" represents traits calculated by cells with one nucleus and without a bud, "A1B" is traits calculated by cells with one nucleus in the mother cell with a bud or the nucleus is dividing at the neck, and "C" is traits derived by cells with one nucleus each in the mother cell and bud. The 405 traits were not independent, and 44 exemplary traits were derived by R package 'apcluster' (negDistMat, r = 2) (Frey and Dueck 2007).

Measure growth rate. Strains were grown in YPD medium to saturation phase at 25°C for two or three days, then diluted 1:100 to 100ul fresh YPD medium at 96-well plate. Two replications of each segregant were placed in the same 96-well plate. The 96-well plates were put on Epoch2 Microplate Spectrophotometer (BioTek) and incubated at 25°C with shaking. The absorbances at 600 nm of each well were determined per hour. The measurements lasted 24 hours and all strains reached saturation phase. The Vmax of growth rate, i.e. the maximum slop of growth curve of each well, was used to estimate the fitness of each strain. To control the positional bias, …

The average normalized values of growth rate were taken as the fitness of each segregant, and listed in Table S2.

Calculate heritability. Because the segregant panel was produced by Bloom et al, broad-sense heritability (H^2), narrow-sense heritability (h^2), additive QTL, and the variance explained by QTL of each morphological trait were calculated by methods consisted with Bloom et al's study (Bloom, et al. 2013). Briefly, H^2 was estimated as $\sigma_G^2/(\sigma_G^2+\sigma_E^2)$, where σ_G^2 was the genetic variance and σ_E^2 was the error variance, which was performed by the 'lmer' function in lme4 R package (Bates, et al. 2015). Narrow-sense heritability was estimated as $\sigma_A^2/(\sigma_A^2+\sigma_{EV}^2)$, where σ_A^2 was the additive genetic variance and σ_{EV}^2 was the error variance. R package rrBLUP was used to calculate h^2 (Endelman 2011). Standard errors of H^2 and h^2 were calculated by delete-one jackknife both.

Additive QTL of each trait was detected using the step-wise forward-search approach developed by Bloom et al (Bloom, et al. 2013). Lod scores for each genotypic marker and each trait were calculated as $-\mathrm{n}(\ln(1-r^2)/2\ln(10))$, where r is the Pearson correlation coefficient between the genotypes and trait values. Significant genetic markers were detected from four rounds using different lod thresholds corresponding to a 5% FDR, which were 2.68, 2.92, 3.72 and 4.9, respectively. A multiple regression linear model was estimated by taken each QTL as independent variables of each trait, and the total phenotypic variance explained by additive QTL was the square of the multiple regression coefficient. Standard tenfold cross validation was performed to derive standard errors. The results were listed in Table S3.

Calculate relatedness to fitness. For each segregant, the average trait values of replications were calculated. For each morphological trait, the raw average values were then scaled into Z-score. Pearson's R between the scaled trait value and the growth rate in YPD medium was used as a proxy of relatedness to fitness for each trait. The results were listed in Table S3.

Calculate CV of replications. Coefficient of variations for each trait were calculated using replications of A11_01 and A11_96, respectively. To evaluate the repeatability of two groups, we use a distance index between two groups of CV as $|CV_{01\_i}-CV_{96\_i}|/(CV_{01\_i}+CV_{96\_i})$, where CV_{01_i} and CV_{96_i} were the value of CV for trait *i* in A11_01 and A11_96, respectively. The results were listed in Table S3.

Define the threshold of narrow-sense heritability. The curve of relatedness to fitness and h^2 was then fitted by a linear equation using 'lm' function in R. The formula was y = 1.0176x + 0.1244, where x was the relatedness to fitness, and y was h^2. When the value of relatedness to fitness was 0.1, the corresponding values of h^2 was 0.226.

Define the threshold of important traits. The 298 morphological traits whose CV distance less than 0.2 were ranked by relatedness to fitness in decreasing order and by mean CV in increasing order, respectively. Then the important traits were defined as the rank indices in both sets less than 150, and the unimportant traits were defined as the rank indices in both sets larger than 149.

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Figure legends

Figure 1 The local adaptive process in a hybrid population. There are three loci (A, B, C) responsible for a trait X. In one population, the states of these three loci of trait X are fixed as (A1, B1, C1), which are (A2, B2, C2) in another population. When this two populations mix again, the states of these three loci in the hybrid offspring need to adapt the new genetic background rapidly. The null model of the relationship between heritability and traits could be one of the three types: non-correlation, positive, or negative. Specific to the three loci, there are numerous combinations of them, which could be summarized to non-additive or additive ways.

Figure 2 Characters of heritability of 405 morphological traits.

(A): The upper is the staining image of yeast cell, in which green circles are cell membrane and blue dots are nucleus; the lower is the schematic diagram of calculating morphological traits.

(B): The distribution of pairwise correlations of 405 morphological traits between different replications of segregant A11_01.

(C): The values of CVs of each morphological traits calculated by replications in A11_01 and A11_96.

(D): The broad-sense heritability (H^2) and narrow-sense heritability (h^2) for each trait. Error bars represent SE, and the dashed line represents $h^2 = H^2$.

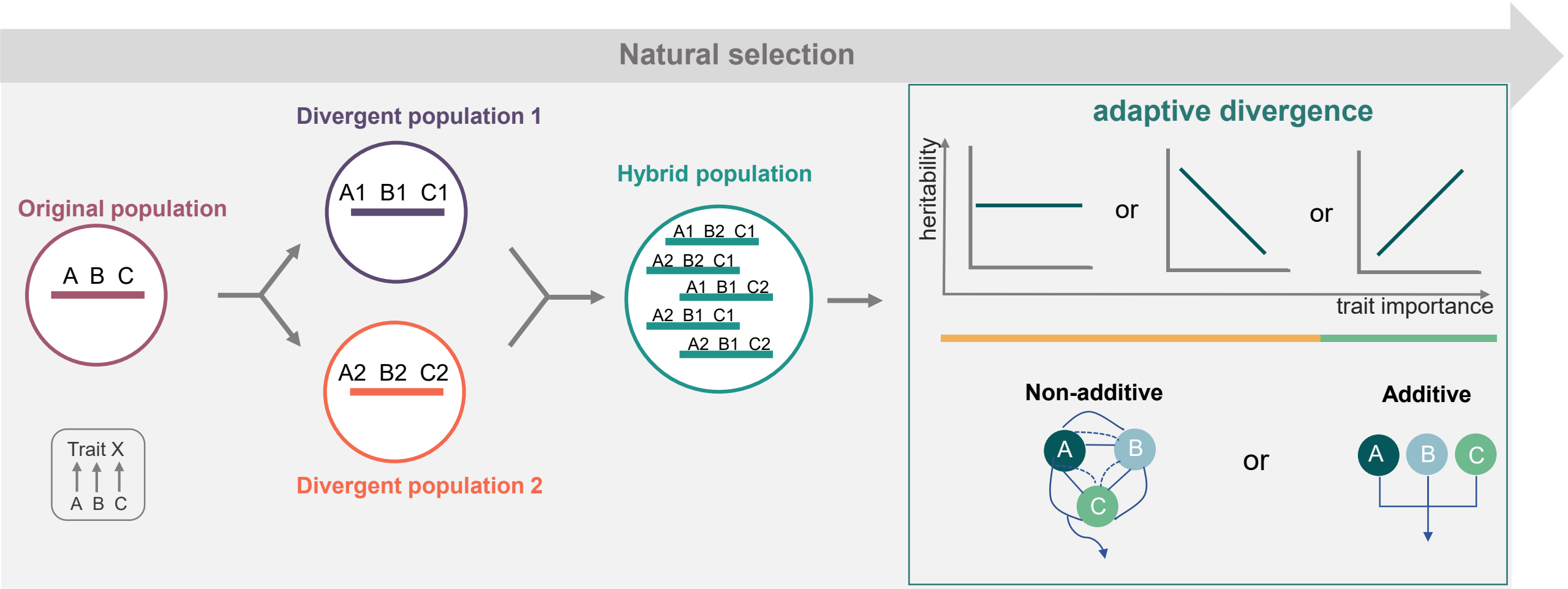
(E): A strong positive correlation between CV_A and CV_R exists (Pearson's $R = 0.802$, $P < 2.2\times10^{-16}$). The gray zone shows the 95% confidence interval of the regression line (blue).

(F): The narrow-sense heritability (h^2) of each trait is plotted against the phenotypic variance explained by additive QTL. Error bars represent SE, and the dashed line represents variance = h^2.

Figure 3 A positive relationship between relatedness to fitness and h^2 for 405 morphological traits.

(A): A strong positive correlation between relatedness to fitness and h^2 is revealed by 405 morphological traits (Pearson's $R = 0.567$, $P < 2.2\times10^{-16}$).

(B): A negative correlation between CV of measurement and h^2 exists in 405 morphological traits (Pearson's $R = -0.33$, $P = 4.35\times10^{-9}$). Error bars represent SE. The blue line is the linear regression line and the gray zone shows the 95% confidence interval.


Natural selection
Original population
A B C
Divergent population 1
A1 B1 C1
A2 B2 C2
Divergent population 2
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Trait X
A B C
adaptive divergence
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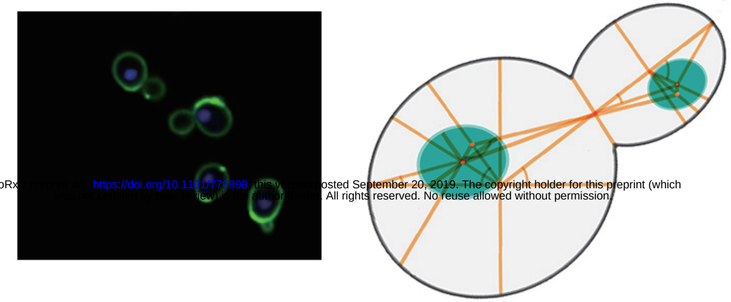
Fig. 1

A



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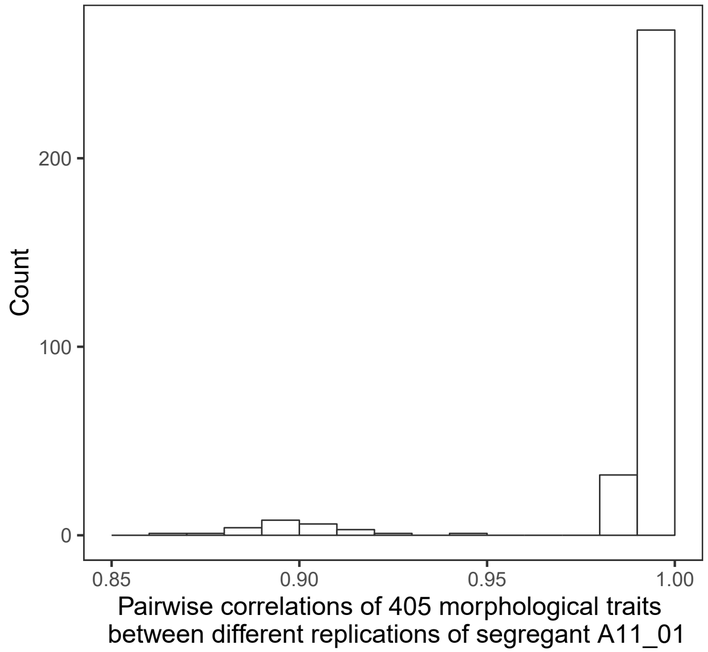

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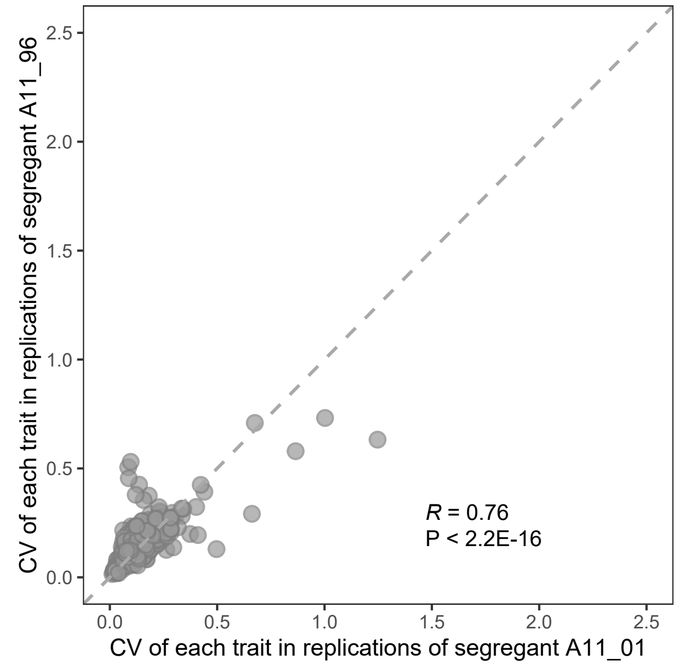

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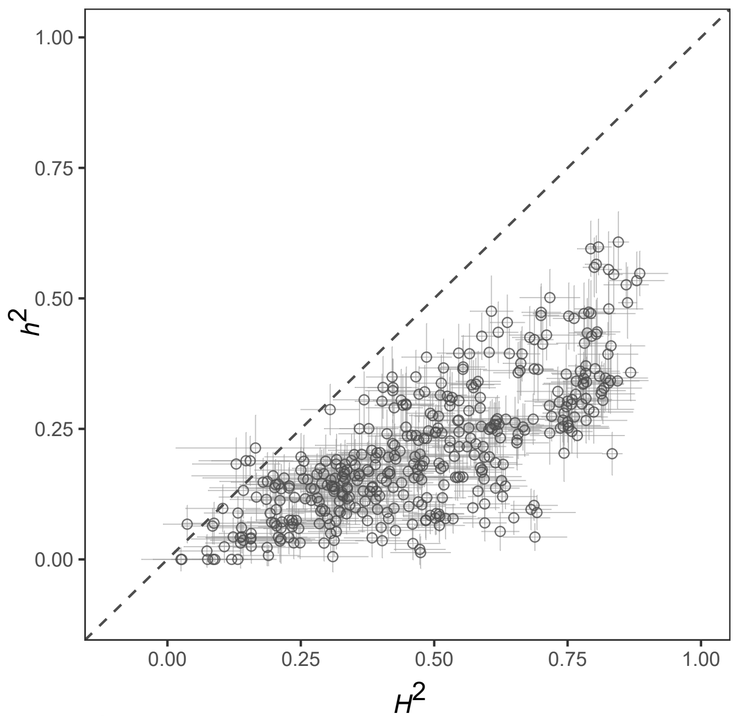
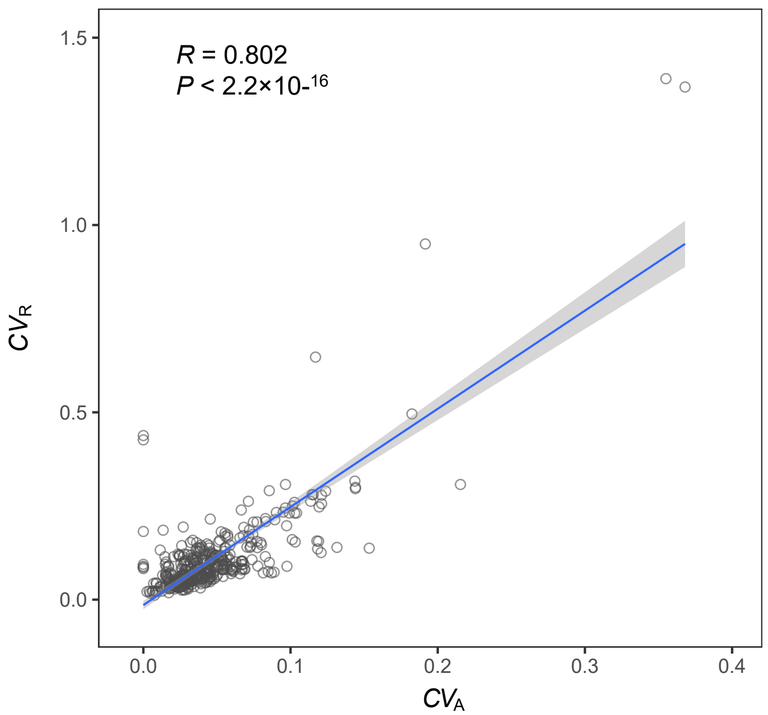


E



F

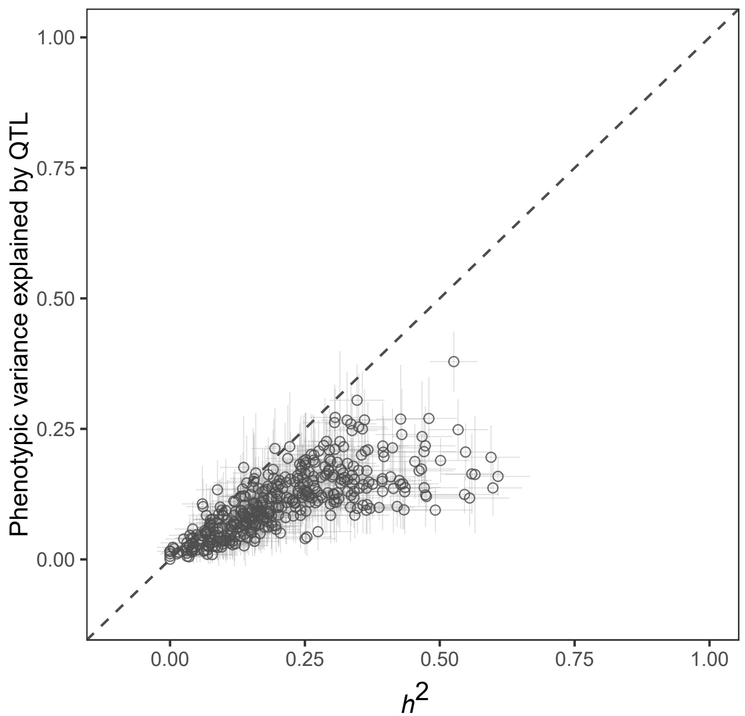
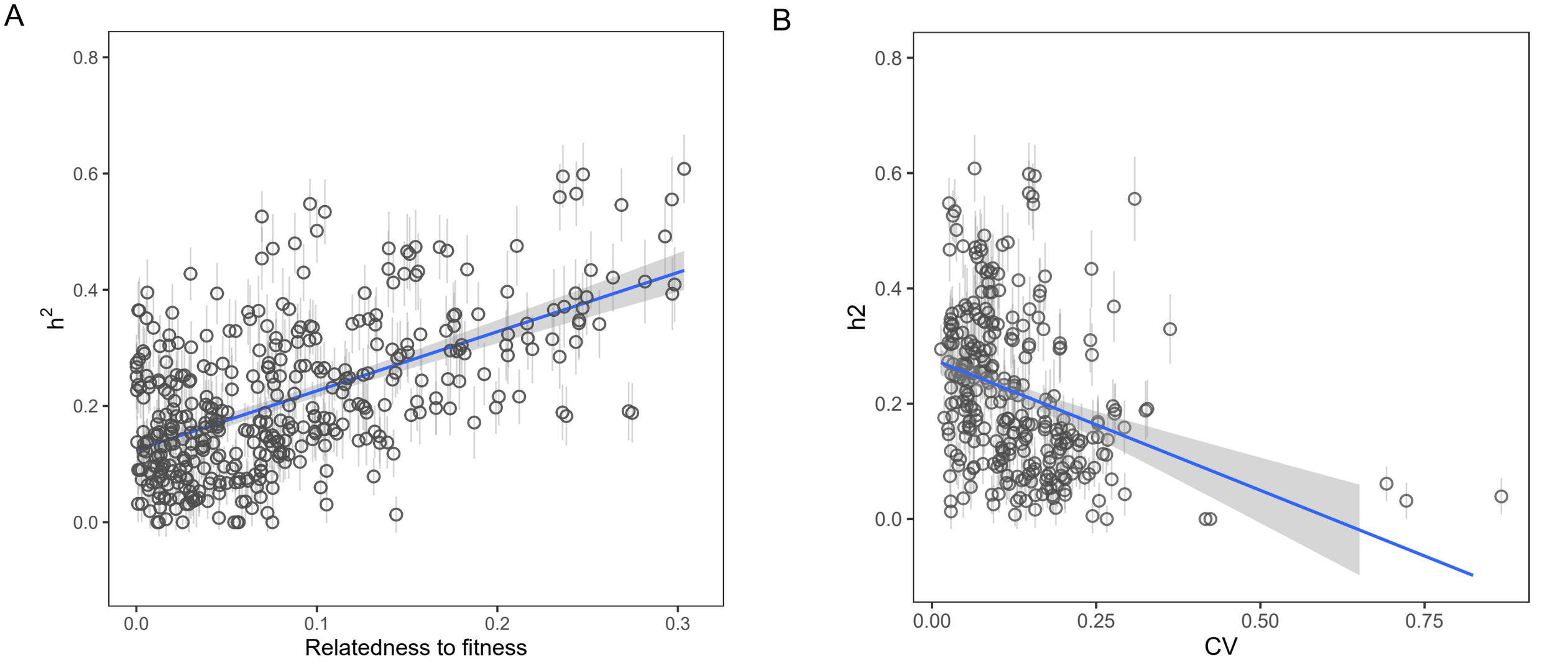


Fig. 2


A
0.8
0.6
0.4
0.2
0.0
h2
0.0
0.1
0.2
0.3
Relatedness to fitness
B
0.8
0.6
0.4
0.2
0.0
h2
0.00
0.25
0.50
0.75
CV