

Natural selection stops the evolution of male attractiveness

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Sexual selection in natural populations acts on highly heritable traits and tends to be relatively strong, implicating sexual selection as a causal agent in many phenotypic radiations. Sexual selection appears to be ineffectual in promoting phenotypic divergence among contemporary natural populations, however, and there is little evidence from artificial selection experiments that sexual fitness can evolve. Here, we demonstrate that a multivariate male trait preferred by *Drosophila serrata* females can respond to selection and results in the maintenance of male mating success. The response to selection was associated with a gene of major effect increasing in frequency from 12 to 35% in seven generations. No further response to selection, or increase in frequency of the major gene, was observed between generations 7 and 11, indicating an evolutionary limit had been reached. Genetic analyses excluded both depletion of genetic variation and overdominance as causes of the evolutionary limit. Relaxing artificial selection resulted in the loss of 52% of the selection response after a further five generations, demonstrating that the response under artificial sexual selection was opposed by antagonistic natural selection. We conclude that male *D. serrata* sexually selected traits, and attractiveness to *D. serrata* females conferred by these traits, were held at an evolutionary limit by the lack of genetic variation that would allow an increase in sexual fitness while simultaneously maintaining non-sexual fitness. Our results suggest that sexual selection is unlikely to cause divergence among natural populations without a concomitant change in natural selection, a conclusion consistent with observational evidence from natural populations.

genetic variance | genetic constraint

Sexual selection has been extensively studied and is known to be both pervasive and strong in natural populations (1, 2). There is evidence that sexually selected traits have diversified more than nonsexual traits (3) and that sexual selection drives phenotypic diversification and speciation (4, 5). Sexually selected traits are associated with high levels of additive genetic variation (6) and have been observed to evolve in response to a change in natural selection (7). It is thus surprising that there are few examples in which the divergence of sexual traits among natural populations of the same species can be attributed primarily to sexual selection (7). Within a single population, strong sexual selection on a heritable trait has been shown to be insufficient to result in contemporary evolutionary change (8), suggesting that sexually selected traits may be subject to an evolutionary limit in nature.

The paucity of examples of contemporary evolution as a consequence of sexual selection is paralleled by a lack of evidence for the efficacy of sexual selection in increasing male mating success under controlled conditions. Although several studies have demonstrated a response to artificial selection on male sexually selected traits (9–12), only one such study, which selected for increased comb size in male domestic chickens (13), resulted in increased male mating success. Selection for increased mating success itself, by allowing female flies to assess male attractiveness, has been successful in one population of *Drosophila melanogaster* after a single generation of selection (14), but allowing female flies to choose mates did not increase male mating success after 3

generations in guppies (15) or after 10 generations in *Drosophila bunnanda* (16).

The potential importance of opposing natural selection in limiting the evolution of male sexually selected traits has long been appreciated (17), but widespread evidence for the antagonistic effects of natural selection has been generally lacking (18, 19), although several studies have provided evidence of natural selection acting against exaggerated sexual traits in relation to specific fitness components [e.g., (20–22)]. Although it can be inferred from these experiments that the natural selection detected may inhibit the further evolution of these traits, the extent of natural selection operating on male traits is difficult to quantify through studies of individual fitness components (23). Evolutionary manipulations of male traits provide a way to determine the relative strength of natural selection that opposes the further evolution of male sexually selected traits.

Here, we demonstrate the existence of an evolutionary limit to selection for an increase in male attractiveness in *Drosophila serrata* and determine the underlying cause of the evolutionary limit. *D. serrata* females discriminate among potential male partners on the basis of a combination of eight male cuticular hydrocarbons (CHCs), which act as contact pheromones (24–26). Sexual selection exerted by female choice causes the evolution of male CHCs in experimental populations (27). Male CHCs are therefore known to be targets of sexual selection in this species, and the directional nature of sexual selection on these traits in multiple populations has been extensively characterized (28). Male CHCs have also been shown to be under reinforcing selection in field populations (29, 30). We implemented artificial index selection for two different combinations of the eight male CHCs (*Materials and Methods* and Table 1). The first combination represented the CHC combination preferred by female flies in this population of *D. serrata*, denoted I_{ss} . To contrast the evolutionary consequences of selecting on the trait combination preferred by female flies, we chose a second combination of CHCs, which was under weak sexual selection (I_{ws}) and was weakly phenotypically correlated with I_{ss} .

Results

After 10 generations of artificial selection, the two replicate lines within each trait combination showed consistent selection responses (Fig. 1A). The I_{ws} trait combination responded to selection to a greater extent (Table 2), consistent with its higher heritability in the base population. Individual CHCs evolved in the direction of selection (compare Tables 1 and 2) for all CHCs in both of the I_{ws} lines, but fewer than half of the individual CHCs evolved in the same direction as selection in the I_{ss} lines, a result expected from the apparent multivariate genetic con-

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Table 1. Vectors of sexual and artificial selection

CHC	β_{2004}	β_{2009}	I_{ss}	I_{ws}	e_{11}
Z,Z-5,9-C _{25:2}	-0.277	-0.108	-0.128	0.659	0.109
Z-9-C _{25:1}	-0.110	0.024	0.004	0.209	0.099
Z-9-C _{26:1}	0.216	-0.016	-0.023	0.052	0.066
2-Me-C ₂₆	-0.194	-0.160	-0.128	0.316	0.185
Z,Z-5,9-C _{27:2}	-0.223	-0.441	-0.291	0.325	0.226
2-Me-C ₂₈	0.752	0.517	0.485	0.528	0.336
Z,Z-5,9-C _{29:2}	0.259	0.544	0.690	-0.116	0.761
2-Me-C ₃₀	-0.380	-0.452	-0.412	0.146	0.444

Directional selection gradients for unstandardized log contrasts of male CHCs were estimated in two separate experiments (β_{2004} and β_{2009}). The two selection indices applied in the artificial selection experiment are I_{ss} and I_{ws} and were constructed on unstandardized traits. The combination of traits (e_{11}) for which the genetic variance changed the most in response to selection was estimated as the first eigenvector of the first eigentensor of the fourth-order genetic covariance tensor.

straint on I_{ss} (26, 31). In addition, the magnitude of the response in individual CHCs was, on average, larger in the I_{ws} than I_{ss} lines for seven of the eight traits (Table 2). Correlated responses in each of the treatments are shown in Fig. S1.

To determine if selecting in the direction of female preference affected male mating success, we subjected male flies from the four selection and two control lines in the selection experiment to competitive mating trials with male flies from the founding stock (Fig. 2). Male flies from the I_{ss} lines had significantly greater mating success than those from the I_{ws} and control lines (generalized linear mixed model: planned contrast $F_{1,3} = 15.37$, $P = 0.030$). Male flies from the control and I_{ws} lines were chosen over stock male flies in significantly fewer than half of the trials (single-sample t test for mean $\neq 0.5$: $t_{3,2} = 21.85$, $P < 0.001$), indicating that the absence of sexual selection in the direction of female preference over the 11 generations had lowered male mating suc-

Table 2. Response of individual CHCs to selection on the I_{ss} and I_{ws} trait combinations

CHC	I_{ss1}	I_{ss2}	I_{ws1}	I_{ws2}
Z,Z-5,9-C _{25:2}	0.016**	0.027***	0.048***	0.038***
Z-9-C _{25:1}	0.026**	0.032**	0.027**	0.137***
Z-9-C _{26:1}	0.002	-0.008	0.011	0.022
2-Me-C ₂₆	0.047**	0.022	0.114***	0.105***
Z,Z-5,9-C _{27:2}	0.026**	0.038**	0.075***	0.067***
2-Me-C ₂₈	0.050***	0.031	0.093***	0.114***
Z,Z-5,9-C _{29:2}	0.292***	0.359***	-0.050	-0.082*
2-Me-C ₃₀	0.054*	0.041	0.107***	0.113***

Values represent the difference in individual trait means between generations 1 and 11, adjusted by the mean change of the control lines, and are shown for each of the two replicate lines for each selected trait combination (t test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

cess. Male flies from the I_{ss} treatment achieved higher mating success than stock male flies, although this was not significantly different from 50% ($t_{1,2} = 2.33$, $P = 0.258$).

The response to selection of the I_{ss} trait combination plateaued, reaching an evolutionary limit after 7 generations (Fig. 1A). In contrast, no selection limit was apparent for the I_{ws} trait combination. Segmented regression (Fig. 1B) indicated break points in the response of both replicate lines of the I_{ss} treatment at generation 7 (Davies test for the breakpoint: $P < 0.001$ for both lines). Breakpoints were also detected in both replicate lines of the I_{ws} treatment (Fig. 1B), but the breakpoints here occurred early during the response to selection and were not associated with an evolutionary limit after the breakpoint. Evolutionary limits can occur from an exhaustion of genetic variance attributable to the fixation of favorable alleles, overdominance at loci responding to selection, or natural selection opposing the response to artificial selection (32). A genetic analysis of the selection responses using the pedigree of all male flies measured during the 11 generations

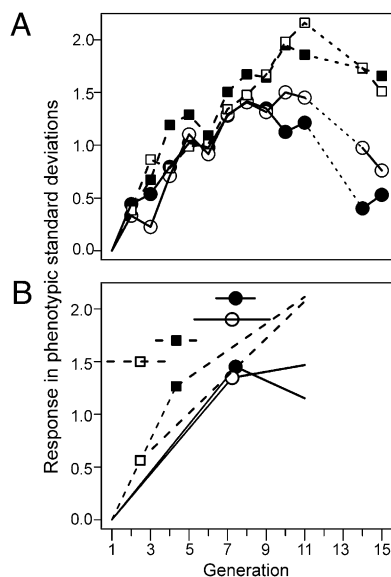


Fig. 1. Direct response of CHC trait combinations to artificial selection. I_{ss} lines are represented by solid lines/circles, I_{ws} lines are represented by dashed lines/squares, and closed/open symbols represent different replicate lines within treatments. (A) Control-adjusted response in phenotypic SDs. During the artificial selection phase (up to generation 11) 60 male flies per line were assayed. All lines were remeasured at generations 14 and 15 using 200–400 male flies per line. (B) Fitted lines from the segmented regression analysis. Breakpoints with 95% confidence intervals are shown as horizontal lines.

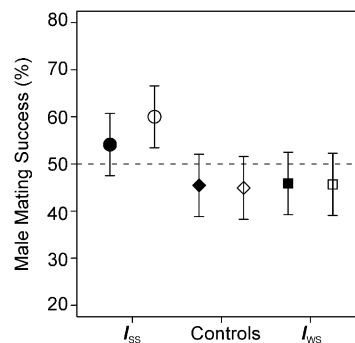


Fig. 2. Mating success of male flies from the selection lines relative to the male flies of the founding stock. After 10 generations of selection, the four selection and two control lines were maintained as random mating populations for 2 generations. Following this, female flies from the founding stock population were allowed to choose between one selection or control line male fly and one founding stock male fly (distinguishable by a small clip to the right wing), with mating success defined by intromission. Between 215 and 220 male flies per line were tested. The significance of the effect of the selection treatments on mating success was tested using a planned comparison [I_{ss} lines (circles) compared with I_{ws} lines (squares) and control lines (diamonds)] under the generalized linear mixed model $Y_{ijk} = T_i + L_{j(i)} + e_{k(ij)}$, where Y_{ijk} is the mating success of the k th individual in the j th line (L) of the i th selection treatment (T). Replicate line was modeled as a random factor nested within the fixed effect of treatment, and binomial errors were modeled using the logit link function. Error bars are 95% confidence intervals. The dotted line represents equal mating success of male flies from the stock and experimental lines.

of selection was used to distinguish among these potential mechanisms.

To test if a depletion of genetic variance during selection explained the evolutionary limit reached by I_{ss} , we determined how the genetic variance changed during selection using an animal model approach (*Materials and Methods*). The trait combination (e_{11}), which experienced the greatest change in genetic variance across all lines during the experiment (Table 1), was closely associated with the change in means of the CHCs in the I_{ss} lines (Table 2) as indicated by the vector correlations of 0.92 and 0.87 between e_{11} and the vector of cumulative change in CHC means for the two replicate I_{ss} lines, respectively. That is, although I_{ws} lines exhibited the greatest change in trait mean, it was the I_{ss} lines in which the genetic variance evolved to the greatest extent. During the first three generations of selection, the average genetic variance of the I_{ss} lines was not significantly different from the average genetic variance of the I_{ws} and control lines ($t_{1,1} = 1.99$, $P = 0.2716$), it but was significantly different by the second ($t_{3,4} = 10.71$, $P < 0.001$) and third ($t_{3,5} = 6.62$, $P = 0.004$) four-generation intervals (Fig. 3A). The average genetic variance of the e_{11} trait combination for the third interval in the I_{ss} lines was 300% of the average of the control and I_{ws} lines in this interval and 658% of the average genetic variance of the control lines in the first interval. The genetic variance in the I_{ss} trait combination itself in the third interval was 160% and 256% greater in the I_{ss} lines than in the control and I_{ws} lines, respectively. No other consistent change in genetic variance across the treatments was found using a more comprehensive investigation of the change in genetic variance in all other trait combinations using the genetic covariance tensor (Fig. S2). A depletion of genetic variance was therefore not responsible for the selection limit, because genetic variance had significantly increased as selection proceeded.

Substantial changes in genetic variance are not expected to accompany changes in mean under the infinitesimal model (33) because each allele has only a small effect, and allele frequency changes that cause a change in genetic variance will be minimal.

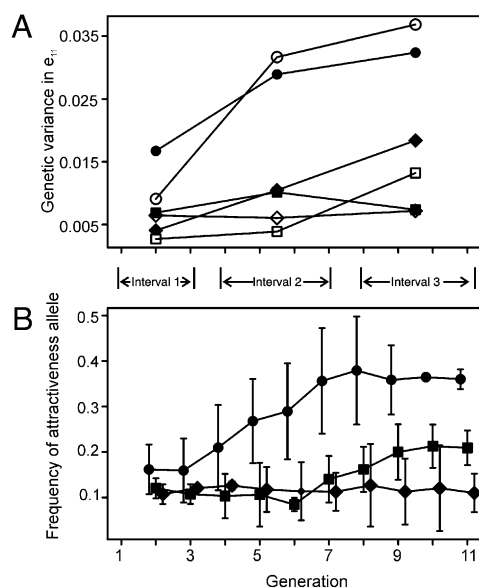


Fig. 3. Genetic analyses of the selection responses. (A) Genetic variance in the trait combination (e_{11}) that exhibited the greatest change in genetic variance among the six selection lines. Genetic variance in e_{11} is shown for I_{ss} (circles), I_{ws} (squares), and control lines (diamonds), with replicate lines within each treatment either closed or filled. (B) Frequency of the major gene inferred from CSA. Error bars are 95% confidence intervals based on the two replicate lines for each treatment.

Therefore, the increase in genetic variance in e_{11} was likely to be associated with genes of major effect. Complex segregation analysis (CSA; *Materials and Methods* and *SI Text*) uncovered the presence of at least one major gene affecting e_{11} (Fig. 3B). The effect size of the major gene can be determined as the difference between the genotypic means for the two homozygotes from the general single-locus model (Table S1) divided by the phenotypic SD of the trait in the first generation in the I_{ss} lines (0.217), and equaled 2.498 phenotypic SDs. The average frequency of the inferred major gene in the control lines remained at around 12%, whereas in the I_{ss} lines, it increased to over 30% by the seventh generation and then remained at around this frequency for the last four generations of the selection experiment. This plateau in inferred allele frequency coincides with the break points estimated for the response to selection in these lines (Fig. 1B), where no response to selection was observed after generation 7. The residual heritability (i.e., that not explained by the major gene) of e_{11} was much higher in the I_{ss} lines ($h^2 = 70\%$) than in the control lines ($h^2 = 9\%$) (Table S2), where h^2 represents polygenic heritability, indicating that there was more than a single gene involved in the response to selection in the I_{ss} lines.

The allele of the major gene that conferred higher male attractiveness was partially recessive (Table S1), indicating that overdominance could not explain why this allele stopped increasing in frequency. After excluding a lack of genetic variance and overdominance of a major gene as possible causes of the evolutionary limit, opposing natural selection is the most likely cause for the lack of continued progress under artificial selection (32). To test for the presence of natural selection, we relaxed artificial selection after the 10 generations and remeasured all populations in generations 14 and 15. The average reduction in the I_{ws} trait in the I_{ws} lines was 21% of the selection response, contrasting with 52% of the selection response in the I_{ss} trait in the I_{ss} lines being lost during this period (Fig. 1A). Because no loss in fertility of male flies from the I_{ss} lines was detected, based on the success of mating pairs during the experiment, this reversion indicates that larval viability selection with a strength equivalent to the 50% truncation artificial selection applied during the experiment acted against an increase in the I_{ss} phenotype.

Discussion

Our experiment has provided an empirical demonstration that selection on male traits in the direction of female preference maintained high male mating success in comparison with control lines and lines selected for a trait combination not preferred by female flies. The alleles resulting in higher male mating success also conferred a substantial fitness cost, however, which was sufficient to counter the strong (artificial) sexual selection, halting the further evolution of the male traits. Mutational studies have tended to find positive covariances between male mating success and other fitness components (34, 35). In contrast, standing genetic variation, which has presumably been sorted by selection, is often characterized by negative genetic covariance among fitness components (36, 37). Our results indicate that under sexual selection, antagonistic pleiotropy between male sexual and nonsexual fitness is likely to evolve, ultimately limiting male trait evolution.

The selection response observed in this study demonstrated that alleles conferring higher male mating success are segregating in this *D. serrata* population, but two lines of evidence indicate that these alleles must segregate at low frequency in the base population, presumably as a consequence of the antagonistic effects on nonsexual fitness inferred from the selection limit. First, genetic variance in the trait combination under I_{ss} is very low compared with those combinations that experience I_{ws} in this (26) and a closely related species (38). This indicates that large amounts of genetic variance are not maintained in the multivariate direction of sexual selection, in contrast to the substantial

levels of genetic variance found for the individual component male traits (6). The response to selection of the individual traits in the I_{ss} lines confirmed the presence of this multivariate constraint, with fewer than half of the individual traits responding in the same direction as the selection gradient in this treatment.

Second, we observed an increase in genetic variance in the I_{ss} lines in response to the artificial selection. The increase in genetic variance could be partially attributed to the increase in frequency of the major gene, but the increase in polygenic heritability indicated that other alleles also increased in frequency under the artificial selection. Male attractiveness therefore exhibits a genetic basis, consisting of favorable alleles at frequencies well below symmetrical frequencies in the base population. In contrast, the I_{ws} lines did not display any consistent increase in genetic variance, suggesting a qualitatively different genetic basis, one that is not dominated by rare alleles. Taken together, these observations suggest that the strength of natural selection on the I_{ss} trait combination results in alleles associated with higher I_{ss} values (and greater sexual attractiveness) being held at low frequencies, which, in turn, results in low genetic variance for increasing male attractiveness in the base population.

The up to sixfold increase in genetic variance of the e_{11} trait combination in the I_{ss} lines relative to the control lines supports a key prediction of quantitative genetic theory that has not previously received strong empirical support (33). The few selection experiments that have been designed to determine if the genetic variance evolves under selection (39–41) have selected on single traits, such as body size, which tend to have higher levels of genetic variance and may only have a weak genetic correlation with fitness (42). Observing changes in genetic variance may therefore require identifying traits under stronger selection than that experienced by individual metric traits.

In conclusion, sexually selected traits of *D. serrata* males, and the attractiveness to female flies that they confer, are held at an evolutionary limit by a lack of the genetic variation that would allow an increase in sexual fitness while simultaneously maintaining high nonsexual fitness. Over a longer evolutionary time scale, divergence in these male sexual traits among nine natural populations of *D. serrata* has been constrained to occur primarily in the direction of greatest genetic variance (g_{max}) rather than in the direction of sexual selection itself (43). In other words, divergent sexual selection has been ineffective in generating divergence in the trait combination preferred by female flies in natural populations. The evolution of these male traits under field conditions might therefore be subjected to the same evolutionary limit that has been identified in the laboratory by this selection experiment. More generally, our study provides a mechanistic explanation for the observation across a range of taxa (7) that sexual selection is unlikely to result in rapid evolution in contemporary populations without an additional ingredient, such as a change in the environment or a change in female preference itself.

Materials and Methods

Selection Indices and Application of Selection. Selection indices are often used in animal and plant breeding to improve a number of individual traits simultaneously (32). We adopted the same approach to select on the basis of eight CHCs in two different directions in the eight-dimensional phenotypic space. Previous work estimated the vector of sexual selection gradients (β) for male CHCs in the Forster stock population of *D. serrata* (26), shown in Table 1 as β_{2004} . Before conducting artificial selection, we reestimated β to determine if linear sexual selection on male CHCs had changed in the 5 y (~120 generations) between this initial estimate and the start of the selection experiment. Using the same experimental design (26), the vector of linear sexual selection gradients was again estimated using the gas chromatograph profiles of 527 male flies from this Forster population (β_{2009} , Table 1). The two β estimates have a vector correlation of 0.855, which was not significantly different from one using a randomization test (Fig. S3), indicating that female preference for male CHCs has persisted in a consistent form under laboratory conditions.

Our original intention in this experiment was to apply artificial selection along two trait combinations: in the direction of β and in the direction of g_{max} . A coding error during the initial application of the linear equations for both trait combinations resulted in selection being applied to two slightly different trait combinations, however. The selection index for the trait combination under sexual selection (I_{ss} , Table 1) was virtually identical to β_{2009} , with a vector correlation of 0.976 between them, and had a standardized univariate linear sexual selection gradient of $\beta = 0.304$. The second vector of linear selection gradients applied in the experiment (I_{ws} , Table 1) was under weak sexual selection ($\beta = -0.053$) and had a weak vector correlation (-0.104) with I_{ss} . At the start of selection, I_{ws} was associated with substantial genetic variance [$V_A = 0.009$, 22.5% of the trace of genetic variance-covariance (G), $h^2 = 26.6\%$], whereas I_{ss} had very low heritability ($V_A = 0.001$, 2.7% of the trace of G , $h^2 = 7.0\%$) as a consequence of a well-characterized multivariate genetic constraint (26, 31) that results in low genetic variance in this direction. Such multivariate genetic constraints cause individual traits to respond in the direction opposite to the selection gradient (42).

Six experimental lines were maintained, two for each of the I_{ss} and I_{ws} treatments and two control lines. For each line, 60 two-day-old *D. serrata* mating pairs were placed in vials for 3 d, after which the sires were killed for gas chromatography (26). Sires were ranked for their I_{ss} and I_{ws} scores, and truncation selection was applied, with two sons and two daughters collected from each of the 30 top-ranked sires for each line. Male and female flies were then randomly assigned into mating pairs from the pool of 2-day-old offspring for the next generation. Control lines were treated in the same fashion but with 30 sires selected randomly. The control lines served two purposes. First, to remove the effects of temporal fluctuations of the line means that commonly occur during long-term selection experiments, the average of the generation means of the two control lines was subtracted from the generation means of the selection lines for all traits (32). Second, the control lines allowed a comparison of the behavior of mean male mating success and genetic variance when sexual selection was prevented from acting without the application of artificial selection in a specific direction.

Measuring the Change in Genetic Variance. To determine how the genetic variance changed during the selection experiment, the data from each selection line were first divided into three intervals, consisting of individuals from generations 1 to 3, generations 4 to 7, and generations 8 to 11. For each interval, only phenotypic data of individuals from that interval were used, whereas the pedigree information for individuals from all intervals was included (44). This resulted in the estimation of eighteen 8×8 G matrices. Genetic variances and covariances pertaining to each selection line and interval combination were estimated using the mixed-model software WOMBAT (45) under the animal model:

$$y = Z_1 u_1 + Z_2 u_2 + e$$

where Z_1 and Z_2 are incidence matrices relating the additive genetic (contained in the vector u_1) and vial (contained in the vector u_2) effects, respectively, to observations in y . The vector e contains the errors. Including the random effect of vial allowed common environment effects experienced by brothers to be estimated. Generation does not appear in the model as a fixed effect because the average of the two control lines within generations was used to correct for generation-specific environment effects (an example of the WOMBAT code is presented in [SI Text](#)).

In applications of index selection, individual trait means may not respond to selection in the direction of their selection gradients (as observed here for the I_{ss} lines, Tables 1 and 2) as a consequence of the genetic covariance structure among traits. Similarly, the evolution of genetic variance of traits cannot be predicted from the selection gradients but, instead, must be determined empirically from the data. Hine et al. (46) showed how changes in genetic variance in multiple traits can be characterized using a fourth-order tensor. This method provides a clear and objective way of determining which trait combinations have experienced the greatest changes in genetic variance. Just as the genetic covariance among multivariate phenotypes consisting of n traits can be described by an $n \times n$ G matrix, the variation among $p \times n \times n$ G matrices can be described by an $n \times n \times n \times n$ tensor, Σ . Eigenanalysis of the tensor yields up to $n(n+1)/2$ or $p-1$ (whichever is smaller) nonzero eigenvalues and their corresponding eigentensors; in this case, the number of G matrices limited the possible number of interpretable eigentensors to 17.

Eigentensors represent $n \times n$ subspaces of genetic variance that vary in size among the p G matrices and can themselves be subject to eigenanalysis to facilitate their interpretation. The leading eigentensor describes the combination(s) of traits that has experienced the greatest change in genetic

variance among the **G** matrices. Here, the leading eigentensor explained 66% of all the variation in genetic variance among the 18 **G** matrices. A single CHC combination, the first eigenvector of the leading eigentensor (\mathbf{e}_{11}), explained 98% of the change in genetic variance that was accounted for by the first eigentensor (i.e., 65% of the total change in genetic variance among the 18 **G** matrices). The change in genetic variance in \mathbf{e}_{11} was associated with the change in CHC means in the I_{ss} lines. To determine whether the increase in genetic variance of \mathbf{e}_{11} in the I_{ss} lines was significant, we tested whether the mean genetic variance of the I_{ss} lines was significantly different from the mean genetic variance of \mathbf{e}_{11} in the I_{ws} and control lines in each interval by conducting two-sided Welch two-sample *t* tests (unequal variances) (47).

CSA. To determine whether the observed increase in genetic variance could be attributed to the changing frequency of a gene of major effect, we conducted a univariate and biallelic CSA (48) on \mathbf{e}_{11} . CSA combines information on known relatives with a mixture model to detect the presence of a major gene and is widely used in the genetic analysis of human populations (48). The CSA was conducted in PAP (49) on data consisting of all individuals from all lines using the mixed model

$$\mathbf{y} = \mathbf{Z}_1\mathbf{u}_1 + \mathbf{Z}_2\mathbf{u}_2 + \mathbf{Z}_3\mathbf{W}\mathbf{m} + \mathbf{e}$$

where the additional term compared with the animal model described above, **Wm**, models single-gene effects. Here, **W** is a matrix consisting of three columns, with rows containing the probabilities of an individual for each of the three possible genotypes of the major gene. The vector **m** contains the genotypic values of the three genotypes, with elements being constrained or allowed to vary depending on the hypothesized genetic effects. The estimation of **Wm** requires information on how the major effect is transmitted from parent to offspring, with different transmission probabilities

associated with genetic or environmental major effects. Three transmission probabilities are either specified or estimated, depending on the model, as detailed below. For example, in the general single-locus model, Mendelian inheritance is assumed and transmission probabilities are set to 1, 0.5, and 0, which are the probabilities of a parent of genotype AA, Aa, or aa transmitting an A allele to its offspring.

Each CSA consisted of the evaluation of five different models (50). Model 1 is the null model, in which no genetic effects are estimated (i.e., all elements of **m** and **u**₁ are equal to zero) and covariance between relatives is allowed to be explained only by the common vial environment experienced by full siblings. Model 2 is the simple polygenic model, in which covariance between relatives is also partitioned into variation attributable to background polygenes but not to a major gene (i.e., elements of **m** are equal to zero). Model 3 is the equal transmission model, in which all parameters are estimated but transmission probabilities are the same for each parental genotype. This model essentially fits a mixture model to the data and implies a major environmental effect (48). Model 4 is the general single-locus model, in which all parameters are estimated except the transmission probabilities, which are set to 1, 0.5, and 0. Model 5 is the general transmission model, in which all parameters, including the three transmission probabilities, are estimated. Significant improvement of this model over a general single locus may indicate shared environmental effects, although it could also reflect non-Mendelian modes of genetic inheritance. The last two models assume Hardy-Weinberg equilibrium. The detailed tests distinguishing among these models are presented in [SI Text](#) and [Tables S1 and S2](#).

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