Effect of the Sex Chromosomes on Trait Dominance in the Shape of the Copulatory System in *Drosophila virilis* × *D. lummei* Progenies

A. M. Kulikov, S. U. Sorokina, A. I. Melnikov, N. G. Gornostaev, and O. E. Lazebny

Koltsov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, 119334 Russia; e-mail: oelazebny@gmail.com

Abstract

The sex chromosomes of the parental species were tested for effect on trait dominance in the shape of the copulatory system in *Drosophila virilis* × *D. lummei* interspecific crosses. The origin of the sex chromosome and the paternal genotype were found to affect the trait dominance in *D. lummei* × *D. virilis* and backcross males heterozygous for autosomes. A correlated variability analysis showed that the two sex chromosomes exert unidirectional effects, shifting dominance towards the conspecific phenotype. The effect of the X chromosome is to a great extent determined by epigenetic factors associated with the paternal genotype.

Introduction

It is clear now that the genetic context affects the degree of dominance for an allele. At the molecular level, dominance is determined by gene expression activity, that is, the functional efficiency of the transcription complex and proteins that regulate the DNA conformational transitions. The specificity of the binding of transcription factors and other accessory proteins to enhancers (DNA sites in the promoter region) further affects the intensity of allele expression. In addition, microRNAs control the timing of mRNA degradation and, consequently, the period of intense expression for particular genes, thus creating, together with transcription factors, an efficient network that regulates genome expression [1, 2]. Negative feedbacks arising between transcription and translation maintain the normal expression level of the target gene within a certain range. It is clear that expression of an allele is apparently determined by the interaction of many components, including both the presence of specific sites in the target gene sequence and the multiplicity of regulatory modules. Thus, a broad network of regulatory interactions is responsible for maintaining expression of a gene and, therefore, a respective trait at an optimal level.

Traits highly involved in the evolutionary process provide an interesting model to evaluate the dominant and recessive relationships. Species divergence with respect to such traits implies a rapid accumulation of evolutionarily significant variations in the genes that control the respective morphological, physiological, and behavioral traits. In the context of evolution, selection at one or several target genes may have two effects:

(1) the genes change in expression level, i.e., epistatic effects change at the levels of transcription, translation, and mRNA maturation; and

(2) the protein product of the gene changes in activity, inevitably leading to selection at the genes that code for partner proteins involved in the same signaling or metabolic cascade.

Note that changes in the concentration of the protein product in case (1) will similarly lead to selection at the genes that code for its partner proteins. By assuming an ancestral, or plesiomorphic, phenotype and an alternative apomorphic phenotype for evolutionarily significant traits, it is possible to assess the structure of the genetic variation associated with the formation of the new phenotype. An accumulation of nonadditive variation associated with the dominant and recessive relationships can be expected according to Fisher's concept of the evolution of dominance [3–5]. Subject to intense debate until recently, Fisher's concept is now commonly accepted and is a component of the views of the evolution of genetic architecture [6, 7].

Multilocus models of selection and mutations, which allow for additive and epistatic gene interactions, have been analyzed comparatively in reviews and original studies [6–10]. The possibility of selection for the alleles that determine epistatic effects of a main locus have been demonstrated for all models. Bagheri and Wagner [11] have analyzed the genetic dynamics of evolution of dominance with traits related to metabolic pathways. Dominance has been shown to depend, in fact, on the duration of selection, i.e., dominant alleles are characterized by more efficient substrate metabolism and, therefore, are far from saturation at their step of the relevant metabolic pathway. Selection for new allelic variants at one-step of the pathway may be caused by mutations that arise in any other step and change the metabolic flux rate. The more significant is the given metabolic flux to survival, the more efficient are the allelic variants of its enzymes and, consequently, the greater is the dominance seen in its status. Thus, all known models allow for an accumulation of dominance with time. At least a substantial part of epistatic regulations is associated with activity of gene expression. Evolution and a gradual increase in dominance in this case agree with Haldane's concept that dominance reflects activity of gene expression and provides a factor of safety to expression [12, 13]. However, it should be noted that all results obtained in model studies need experimental verification.

We have previously shown that the male copulatory organ is the most rapidly evolving morphological structure in 11 closely related *Drosophila* species of the *virilis* group [14]. Interspecific hybrids have been examined to evaluate the degree of dominance for the parental phenotypes. The analysis has identified the phenotype of the youngest species *D. novamexicana* as the most recessive and that of the evolutionary ancient species *D. virilis* as the most dominant

[15]. In this work, interspecific crosses *D. virilis* × *D. lummei* and backcrosses of hybrid males with *D. virilis* were performed to estimate the role that the sex chromosomes play in determining the degree of dominance for the *D. virilis* and *D. lummei* phenotypes of the male copulatory organ.

Materials and Methods

Strains *D. lummei* 200 (Serebryanyi Bor, Moscow) and *D. virilis* 160 were from the *Drosophila* strain collection of the Institute of Developmental Biology and carried the following recessive autosomal markers: *broken* (*b*) on chromosome 2, *gapped* (*gp*) on chromosome 3, *cardinal* (*cd*) on chromosome 4, *peach* (*pe*) on chromosome 5, and *glossy* (*gl*) on chromosome 6. The first two markers are expressed as gaps in the posterior crossvein and longitudinal vein 2, respectively; the other markers determine different eye colors, which are well identifiable visually. F_1 hybrid males were obtained from crosses of *D. lummei* males with *D. virilis* females and reciprocal crosses of *D. virilis* males with *D. lummei* females. In addition, F_b males were obtained by backcrossing hybrid males with *D. virilis* females. Crossing schemes are shown below.

1) Direct crosses

   P ♂ $X_{Lu}Y_{Lu}$, $A_{Lu}A_{Lu}$ × ♀ $X_{Vi}X_{Vi}$, $A_{Vi}A_{Vi}$

   → F_1 ♂$X_{Vi}Y_{Lu}$, $A_{Vi}A_{Lu}$ (hereinafter abbreviated $F_1X_{Vi}Y_{Lu}$)

   ♂ F_1 $X_{Vi}Y_{Lu}$ $A_{Vi}A_{Lu}$ × ♀ $X_{Vi}X_{Vi}$, $A_{Vi}A_{Vi}$

   → F_b ♂$X_{Vi}Y_{Lu}$, $A_{Vi}A_{Lu}$ (abbreviated $F_bX_{Vi}Y_{Lu}$)

2) Reciprocal crosses

   P ♂ $X_{Vi}Y_{Vi}$, $A_{Vi}A_{Vi}$ × ♀ $X_{Lu}X_{Lu}$, $A_{Lu}A_{Lu}$

   → F_1 ♂$X_{Lu}Y_{Vi}$, $A_{Vi}A_{Lu}$ (abbreviated $F_1X_{Lu}Y_{Vi}$)

   ♂ F_1 $X_{Lu}Y_{Vi}$, $A_{Vi}A_{Lu}$ × ♀$X_{Vi}X_{Vi}$, $A_{Vi}A_{Vi}$

   → F_b ♂$X_{Vi}Y_{Vi}$, $A_{Vi}A_{Lu}$ (abbreviated $F_bX_{Vi}Y_{Vi}$)

   A, autosomes

Backcross males heterozygous at all autosomes were selected for further analysis.

All crosses were carried out at 25°C; a standard food medium and glass tubes of 22 mm in diameter were used; the progeny density was 50–70 flies per tube.

Analysis of morphological structures: The mating organ was dissected using thin steel needles in a drop of water under a binocular microscope at a magnification of 12×8. To remove adipose structures, preparations were incubated in boiling 10% NaOH.

A total of 121 samples were examined, including 14 from *D. lummei* males, 13 from *D. virilis* males, 25 from F_1 males obtained by crossing ♂ *D. lummei* × ♀ *D. virilis*, 31 from F_1 males obtained by crossing ♂ *D. virilis* × ♀ *D. lummei*, 10 from F_b males with the genotype X_{Vi} Y_{Vi}, A_{Vi} A_{Lu}, and 28 from F_b males with the genotype $X_{Vi}Y_{Lu}$, A_{Vi} A_{Lu}.

Morphometry was carried out using organ images, which were obtained using a Jen-100C electron microscope in the scanning mode at an accelerating voltage of 40 kV and an instrumental magnification of 300–500×. The sagittal view of the phallus was conventionally divided into four areas: the aedeagus body, gonites, apodeme, and cook. A coordinate grid was superimposed on the image. A conventional point at the junction of the aedeagus area, gonites, and apodema (hereinafter referred to as the central point) was used as a landmark. Morphometric parameters (MPs) were obtained as distances between the intercrosses of coordinate lines with each other and the image outline. A scheme of measurements is shown in Fig. 1.

Coordinate axes were drawn through three main points that were readily identifiable in all images: point 1 marked the basal region of the junction of the aedeagus, gonites, and apodeme; point 2 marked the cook base at the top of the aedeagus; and point 36 was at the end of the apodeme. The two axes drawn through points 1 and 2 (MP 1) and 1 and 36 (MP 28) were divided into four sections, which showed the aedeagus and apodeme points whose distances to point 1 corresponded to 70.0, 50.0, 25.0, and 0.0% of the total length of the respective axis (points 9, 12, and 15 for the aedeagus and 37, 40, and 43 for the apodeme). Point 6 was additionally identified for the aedeagus as a point that is 93.75% of the MP 1 length away from point 1. A vertical axis was drawn through point 6, and the MP 2 axis was drawn at its half-length and was parallel to MP 1. MP 2 showed the maximum aedeagus length, which was determined by the bent of the distal part of the outline. A coordinate grid for gonite traits was oriented according to the aedeagus coordinate grid. Point 27 marked the left margin of the gonite base; the MP 23 axis went down from point 27 orthogonally to MP 1 to the interception with the gonite outline (point 28). Point 29 was at a half-length of MP 23. Horizontal axes passed through points 28 and 29 to the interceptions with the paramere outlines. Point 32 was at the half-length of the segment between points 28 and 35, and the vertical axis drawn through point 32 showed the gonite width. Points 19–26 marked the maximum bents of the dorsal and ventral parts of the aedeagus outline. MPs and the corresponding points of intersection between coordinate lines and the aedeagus outline are summarized in Table 1. The angles characterizing the inclinations of the cook and apodeme (MP 2 and MP 28 axes) relative to the MP 1 axis were designated α and β and expressed in radians.

To exclude the scale effect, PM indices (MPIs) were calculated according to a method used previously to evaluate the inter- and intraspecific variations of the genitals in the *virilis* species group (1). MPIs were obtained as MP-to-MP 1 ratios and were numbered according to the numbers of respective MPs. The traits expressed in radians were included in the analyses without normalization.

Statistical analyses were carried out using Statistica 10 software (Statsoft, United States). Trait dominance was evaluated in interspecific crosses by comparing the trait variation for the parental strains and hybrids; the significance of differences was assessed by an analysis of variance; and groups homogenous in terms of the variation of a particular trait were identified using post hoc comparisons and Tukey's test for unequal sample sizes. The generalized linear model (GLM) was used to evaluate the effect on a particular trait for the sex chromosomes, the paternal genotype, and their pairwise combinations. To account for multiple testing, the Holm–Bonferroni correction was used in all significance tests. A factor analysis was used to study a covariance of traits; factor loadings were estimated by rotating principal component axes via a normalized varimax procedure.

Results

One-way ANOVA was used in sample comparisons. A difference in particular trait between progeny of interspecific crosses and one or both of the parents made it possible to speculate about trait dominance in the hybrid. The logic of estimating the degree of dominance for a trait has been described previously [15]. Based on the distribution of the hybrid and parental genotypes over groups isolated by post hoc comparisons, it is possible to identify the following variants: incomplete dominance; dominance of the *D. virilis* phenotype, the *D. lummei* phenotype, or a new phenotype (N, when the parental phenotypes do not differ from each other, but differ from the hybrid one); and lack of differences among all phenotypes. We used two categories, dominance (D) and overdominance (oD), in order to more accurately evaluate the degree of dominance. The resulting data clustering variants were ranged by the degree of phenotype dominance. Because each genotype might occur in more than one homogeneous group, all cases where a genotype in question appears together with the parental genotypes in at least one group were considered to suggest no significant difference (ns) in order to evaluate the degree of dominance more rigorously. The variants $f_{1(b)}<l<v$, $f_{1(b)}<l\leq v$, $v<l<f_{1(b)}$, and $v\leq l<f_{1(b)}$ suggested overdominance of the *D. lummei* phenotype (oD_{Lu}); the variants $l,f_{1(b)}<v$, $f_{1(b)}\leq l<v$, $f_{1(b)},l<v$, $v<l,f_{1(b)}$, $v<l\leq f_{1(b)}$, and $v<f_{1(b)},l$, dominance of the *D. lummei* phenotype (D_{Lu}); $l<f_{1(b)}<v$,

$l \leq f_{1(b)} < v$, $l \leq f_{1(b)} \leq v$, $v \leq f_{1(b)} \leq l$, $v < f_{1(b)} \leq l$, and $v < f_{1(b)} < l$, intermediate dominance (ID); $f_{1(b)}, v < l$, $v, f_{1(b)} < l$, $l < f_{1(b)}, v$, and $l < v, f_{1(b)}$, dominance of the *D. virilis* phenotype (D_{Vi}); and $l < v < f_{1(b)}$, $l < v \leq f_{1(b)}$, $f_{1(b)} < v < l$, and $f_{1(b)} \leq v < l$, overdominance of the *D. virilis* phenotype (oD_{Vi}).

Table 2 summarizes the results of MPI comparisons for males of the parental strains, male progenies of direct and reciprocal crosses of the parental strains, and the backcross males that were similar to F_1 males in being heterozygous at all autosomes. MPIs 3, 21, 22, 23, 25, 27, 29, and 31 did not significantly depend on the genotype and are not included in Table 2. The MPIs that showed significant correlations to each other were grouped; the results of this grouping are considered below, following the results of factor analyses. Here we describe the most general results of the ANOVA. First, in all genotypes, traits at which the *D. virilis* phenotype dominated were slightly more numerous than those at which the *D. lummei* phenotype dominated. The majority of traits showed different degrees of dominance in different genotypes, and only 5 (MPIs 5, 7, 13, 16, and 26) out of the 27 MPIs displayed the same degree of dominance in all genotypes. MPI 13 did not significantly differ between the parents and progenies in all crosses, but significantly differed between F_1 males from both direct and reciprocal crosses and F_b $X_{Vi}Y_{Vi}$ males. In contrast, four MPIs (2, 15, 28, and 34) showed a variation in dominance from insignificant differences between progenies and both of the parental genotypes to distinct dominance or even overdominance of one of the phenotypes. Nine traits (MPIs 8, 11, 18, 19, 20, 24, 30, and 33 and β) showed the greatest variation of dominance ranks, from dominance of the *D. virilis* phenotype to dominance of the *D. lummei* phenotype. Given that all F_1 and F_b genotypes were similarly heterozygous at all autosomes, the origin of the sex chromosomes and the identity of the male parent were likely to substantially affect trait expression.

The genotype of F_1 males obtained by crossing ♂ *D. lummei* 200 × ♀ *D. virilis* was $X_{Vi}Y_{Lu}$ $A_{Vi}A_{Lu}$, the same as in the F_b males that were obtained by backcrossing these F_1 males with *D. virilis* females and were heterozygous at all autosomes. Crossing over is suppressed in heterozygous males, and genetic material is not exchanged between *D. virilis* and *D. lummei* homologous chromosomes. However, changes in the degree of dominance were observed for 12 out of the 27 traits. The finding supports the assumption that epigenetic factors determined by the paternal genotype (the ♂ *D. lummei* genotype for the F_1 males and the heterozygous ♂ F_1 genotype for the F_b males) exerted their effect on expression.

To evaluate the effects of the sex chromosomes, the paternal genotype, and their combination, the GML method was used to analyze the $F_1X_{Vi}Y_{Lu}$, $F_bX_{Vi}Y_{Lu}$, $F_1X_{Lu}Y_{Vi}$, and $F_bX_{Vi}Y_{Vi}$ genotypes identical in autosome composition. Given the incomplete design of crosses and, therefore, the incomplete design of ANOVA, the sex chromosomes, the paternal genotype, and

their pairwise combinations were tested for effect independently. The results are summarized in Table 3. In total, 16 traits were found to depend on the factors in question.

The most significant effects on the traits were observed for the paternal genotype (15 traits: MPIs 2, 8, 10, 11, 13, 17, 19, 20, 26, 30, 32, 33, and 34 and the angles α and β) and a combination of the paternal genotype and the X chromosome (8 MPIs: 10, 11, 18, 19, 20, 30, 32, and 34). The traits affected by the factors are uniformly distributed over the three phallus structures, determining the shapes of the distal, central, and basal parts of the aedeagus; the gonite length; and the apodeme bent. A combination of the Y chromosome and the paternal genotype was not found to significantly affect the phallus shape.

Paternal genotype-independent effects of the sex chromosomes were far weaker. The X chromosome was shown to significantly affect four traits (MPIs 2, 10, and 13 and α); the Y chromosome, three traits (MPIs 11 and 18 and α); and their combination, two traits (MPI 18 and α). The traits characterized the shapes of the central and distal parts of the aedeagus and the position of the cook on the aedeagus end.

Diverse changes in dominance, including both shifts towards the *D. virilis* phenotype and those towards the *D. lummei* phenotype, were observed for the traits under study in genotypes differing in the origin of the sex chromosomes. The pattern was further complicated because the paternal genotype affected trait expression, which often changed oppositely in response to similar sets of factors. To observe regularities in the changes, we performed factor analyses and identified groups of traits that varied in a correlated manner.

A factor analysis was carried out for the pooled sample, including the four hybrid and two paternal male genotypes. The number of factors was selected based on factor values, which were to be no less than unity. Ten factors were extracted in total and together accounted for 72.2% of the variation observed. A principal component analysis was used to study correlated variability. Factor loadings were obtained by normalized varimax rotation, and those with weights higher than 0.5 are summarized in Table 4. Almost each of the factors combined topologically close traits that determined the variation of structurally integrated parts of the copulatory organ.

Factor 1 included the traits that are related to the apodeme bent angle relative to the MP 1 axis and the characteristics of apodeme curvature; factor 2, those that reflect the shape of the ventral part of the aedeagus projection; and factor 3, the traits that are related to the height of the apodeme outline. Factor 4 determined two topologically close characters, the height of the aedeagus and the curvature of its ventral surface at a distance of 25% of the aedeagus length from its base. Factor 5 included the traits that characterize the shapes of the ventral and basal

regions of the gonite outline; factor 6, the traits that characterize the gonite width at the base and the height of the ventral curvature of the aedeagus at its distal end. Factor 7 included the traits that reflect the cook position at the end of the aedeagus and the aedeagus outline over the cook. Factor 8 included the traits that characterize the gonite length and the species-specific shape of ventral and dorsal bents in the aedeagus projection; these traits are the most variable evolutionarily. Factor 9 included the traits that characterize the aedeagus height in the central region; and factor 10, the height of the gonite projection.

Using the factor estimates as new variables for each specimen, we repeated one-way ANOVA for all samples. A significant effect of the genotype on the variation of the new variables was not observed for factors 3, 5, and 10. For the other factors, the results of post hoc comparisons are summarized in Supplementary 1.

Latent features of the sex chromosomes and the paternal genotype were tested for effect by GLM with the factor estimates that were obtained after excluding the paternal genotypes from the analysis, as in the case of individual traits. A new structure included 10 factors, which together accounted for 71% of the variation observed for the heterozygous genotypes. In the case of new factors 1, 3, 5, 6, and 9, the factor loadings remained the same in terms of the variables included with weights of more than 0.5. MPIs 18 and 19 were included in new factor 4 rather than factor 2 as in the previous analysis. Of the factor loadings of previous factor 8, MPIs 5 and 7 were included in new factor 7 and MPI 26 was included in new factor 10. However, topological proximity was seen again for traits included in new factor loadings.

The effects of the sex chromosomes, the paternal genotype, and their pairwise combinations on the new variables were confirmed for factors 1, 4, 7, 9, and 10. Significant effects were established for the paternal genotype ($F=15.7$, $p<0.001$) and a combination of the paternal genotype and the X chromosome ($F=16.4$, $p<0.001$) in the case of factor 1; the Y chromosome ($F=9.3$, $p=0.023$), the paternal genotype ($F=18.2$, $p<0.001$), and a combination of the paternal genotype and the X chromosome ($F=40.3$, $p<0.001$) in the case of factor 4; the X chromosome ($F=30.0$, $p<0.001$), the Y chromosome ($F=15.1$, $p=0.002$), the paternal genotype ($F=19.8$, $p<0.001$), and a combination of the sex chromosomes ($F=20.9$, $p<0.001$) in the case of factor 7; the X chromosome ($F=11.8$, $p<0.001$), the Y chromosome ($F=9.8$, $p=0.020$), the paternal genotype ($F=9.5$, $p=0.001$), and a combination of the sex chromosomes ($F=11.9$, $p=0.007$) in the case of factor 9; and the paternal genotype ($F=6.6$, $p=0.012$) in the case of factor 10.

A detailed comparison of the results obtained for the sex chromosome origin and paternal genotype dependencies of individual and latent traits is given in Supplementary 2. To

summarize, the two sex chromosomes and the identity of the male parent affect the degree of dominance for traits characterizing the shape of the copulatory organ in interspecific crosses of *D. virilis* and *D. lummei*. Figure 2 provides a scheme of how the degree of dominance of copulatory organ traits changed in hybrid progenies and what roles the sex chromosomes and the male parent identity played.

Changes in dominance were most often associated with the identity of the male parent and a combination of this factor with the origin of the X chromosome. According to the analysis of correlated variability, a substitution of the *D. lummei* X chromosome present in F_1 $X_{Lu}Y_{Vi}$ males with the *D. virilis* X chromosome in F_b $X_{Vi}Y_{Vi}$ males greatly decreased the degree of dominance of the *D. lummei* phenotype, which was not expressed in the backcross males as far as their latent traits are concerned.

Weaker effects on trait expression were observed for the sex chromosomes taken separately or in combination. Independent effects of the Y chromosome and combined effects of the X and Y-chromosomes were observed in the analysis of individual traits far less frequently than in the analysis of factor structures, which reflected the general correlated variation of the primary traits. It is possible to assume that weak, but unidirectional effects of the Y chromosome come to be better seen when the correlated variation is analyzed. A substitution of the *D. lummei* Y chromosome in F_b $X_{Vi}Y_{Lu}$ backcross males with the *D. virilis* Y chromosome in F_b $X_{Vi}Y_{Vi}$ backcross males led again to a great decrease in the degree of dominance of the *D. lummei* phenotype, which was not expressed in latent traits of the backcross males.

Note additionally that the traits that characterize the position of the top of the aedeagus and are the most evolutionarily contrast showed intermediate dominance with no appreciable dependence on the sex chromosomes or the parental male identity.

Discussion

Traits of the genitals, including the male copulatory organ, stand apart from other morphological structures. Sexual selection targets not only the genes involved in regulating gametogenesis, sexual behavior, and physiological conditions for fertilization (seminal fluid proteins), but also the gene set that controls the shape of the male genitals, which evolves most rapidly among all morphological traits [16]. Thus, the structure of the male genitals is often a main, if not the only, taxonomically important trait suitable for distinguishing sibling species in the orders Coleoptera [17–24] and Diptera of the class Insecta. A higher rate of accumulating interspecific differences

makes it possible to assume that genital traits are subject to directional or disruptive selection during speciation and stabilizing selection in the steady-state period of species life.

How does the shape of the male genitals come to be a target of selection? Certain progress in understanding the problem has been achieved in experiments with *Drosophila*. Jagadeeshan and Singh [25] have found that mating behavior in the closely related species *D. melanogaster* and *D. simulans* is directly associated with the species-specific shape of the posterior lobes of the genital arch and the cerci, which are external structures of the male sexual system. Their experiments have shown that the time course of copulation depends on the genital coupling mechanics, which is determined by the male external genitals. The final stage of tight genital coupling was 2–5 times shorter, while the preliminary stage of unstable coupling was prolonged in the case of heterospecific mating. The findings suggest incomplete insemination of females for heterospecific copulations and indicate that a certain mechanism makes a copulating pair sensitive to the specificity of their contact. Hoikkala and colleagues [26] have analyzed the within-population variation of copulation duration in *D. montana* and have associated the duration of the first copulation with female propensity to remate. We have shown that sensory microchaetae occurring on the ventral surface of the aedeagus mediate the association of evolutionarily significant parts of the copulatory system with mating behavior in *Drosophila* [27].

Both mating behavior and copulatory organ shape are traits that are not directly related to adaptation and, as such, are subject to apparent stabilizing selection [28, 29] in populations approaching their optimal fitness. In other words, selection affects these traits by acting through adaptations present in particular groups of individuals. Because the traits are specifically expressed as predictors of reproductive efficiency, this selection apparently acts as sexual selection. The selection type changes to directional or disruptive when a new adaptation develops or the adaptive norm changes rapidly, remaining indirect.

As far as the genetic variation is considered, its additive, dominance, and epistatic components may contribute to the development of a new phenotype. Our experiment was not designed to exactly determine the variance components. However, given that the autosome sets were identical in the males under study, the fact that the degrees of dominance of the parental phenotypes differed between males with different sex chromosome sets indicates that epistatic effects associated with the parental male identity and regulatory effects of the X and Y-chromosomes account for the changes in dominance. Neither primary nor latent traits remained constant in phenotypic expression across all genotypes obtained via interspecific hybridization and backcrossing.

The analysis of individual traits did not give grounds to state that the modifying factors in question considerably shift the phenotype of heterozygous males towards one of the parental phenotypes. The portions of traits where the *D. virilis* or *D. lummei* phenotype dominated were much the same, although substantial changes were observed in various regions of the morphological structure of the copulatory system. However, the analysis of correlated variability indicated that dominance is associated with conspecificity of the sex chromosomes. The finding that the *D. virilis* phenotype increased in expression in males carrying the sex chromosomes of the same species may be associated with the additive genetic variance determined by structural genes of the *D. virilis* X chromosome. For instance, Carson and Lande [30] have shown that sex-linked genes are responsible to the extent of 30% for the development of an extra row of cilia on the tibia of males as an evolutionarily new secondary sexual character in a natural *Drosophila silvestris* race [30].

Orr and Betancourt [31] have proposed a mutation-selection balance model to explain how a recessive variation at adaptive traits may accumulate in populations subject to directional selection. They have shown that Haldane's sieve does not hold when the genetic variation pre-existing in a population provides a source for selection and the direction of selecting polymorphic alleles changes in response to environmental changes, the probability for a mutation to be fixed being independent of its dominance. The results [31] make it possible to think that the advent and fixation of a new dominant mutation is a rare event in species evolution. Contrarily, when the persisting recessive variation is fixed to produce a new phenotype, the effect is opposite; i.e., the phenotype of the evolutionarily older species comes to dominate.

It is still poorly understood how the sex chromosomes affect the inheritance of evolutionarily significant traits in interspecific hybrids, including the traits that are involved in prezygotic mechanisms of species isolation. Yet there are data that support a significant regulatory effect of the two sex chromosomes on expression of autosomal genes. Trans-regulatory factors substantially affect expression of X-chromosomal genes in hemizygous *D. simulans* males [32], and trans and combined cis–trans effects of the X chromosome on cross-genome expression activity are second only to trans-regulatory activity of chromosome III in *D. melanogaster* males [33]. As for the traits that contribute to isolation barriers, the most comprehensive data are available for sterility traits. Mechanisms responsible for male sterility in interspecific hybrids can be considered as a model of interactions between autosomes and the sex chromosomes. A *Stellate* sequence cluster is in the *D. melanogaster* X chromosome and codes for a homolog of the β subunit of protein kinase CK2; its overexpression results in male sterility. The Y-linked *Su*(*Ste*) repeats act via RNA interference to prevent *Stellate* overexpression from [34, 35]; i.e.,

male fertility strongly depends on the interaction of the X and Y chromosomes. A similar mechanism regulating male fertility involves the Odysseus-site homeobox protein (OdsH), which is encoded by the X chromosome in species of the *melanogaster* group. OdsH binds to heterochromatic sequences of the Y chromosome. A combination of the X and Y-chromosomes from different species in one hybrid often causes decondensation of Y-chromosomal chromatin, dramatically distorts expression of autosomal genes, and leads to sterility [36, 37]. This genetic system provides another example of interactions between the X and Y-chromosomes.

It is of interest that a substantial role of epistatic interactions between the X chromosome and autosomes has been demonstrated for the species-specific pattern of male courtship songs in the *D. montana* phylad, another trait that contributes to prezygotic barriers [38].

An important role that the Y chromosome plays in regulating the phallus shape traits in interspecific hybrids is likely to be directly associated with trans-regulatory activity. The Y chromosome harbors only 23 single-copy protein-coding genes, 13 of which are expressed exclusively in the testis and are mostly related to hybrid male sterility [39–42]. The additive variation of other ten genes can only make a negligibly small contribution to the phenotype at quantitative traits of morphological structures. Yet there is experimental evidence for epistatic interactions of Y-chromosomal sequences with the X chromosome and autosomes. For instance, when activity has experimentally been evaluated for the complex of male-specific lethal proteins and roX1,2 RNAs, which is responsible for dosage compensation of X-linked genes in *Drosophila* males, the Y chromosome has been found to affect the survival of *roX1 roX2* mutant males, the effect depending on the source of the Y chromosome [43]. Male survival was low in the case of paternal transmission of the Y chromosome and high in the case of its maternal transmission. The findings indicate that the Y chromosome modulates dosage compensation by acting through roX1/roX2-mediated modification of heterochromatin [44] and/or recognition of the X chromosome by the entire male-specific lethal protein/roX1,2 RNA complex. In addition, the findings support our observation of a distinct paternal effect, which is seen independently in expression of the majority of traits or acts in combination with the effect of the X chromosome. Finally, Lemos et al. [45] have provided direct evidence for regulatory activity of the Y chromosome in experiments on differential genome activity influenced by the Y chromosome.

Thus, our analysis of correlated variability showed that the two sex chromosomes exert unidirectional effects, shifting dominance towards the conspecific phenotype. The effect of the X chromosome is largely determined by epigenetic factors associated with the paternal genotype.

References

1. *Hobert O.* Gene Regulation by Transcription Factors and MicroRNAs // Science. 2008. V. 319. № 5871. P. 1785-1786.
2. *Qiu C., Wang J., Yao P., Wang E., Cui Q.* MicroRNA evolution in a human transcription factor and microRNA regulatory network // BMC Systems Biology. 2010. V.4. №90. P. 1-8, http://www.biomedcentral.com/1752-0509/4/90
3. *Fisher R A.* The possible modification of the response of the wild type to recurrent mutations // Amer. Nat. 1928a. V.62. №679. P. 115–126.
4. *Fisher R A.* Two further notes on the origin of dominance // Amer. Nat. 1928b. V.62. №683. P. 571–574.
5. *Fisher R.A.* The Genetical Theory of Natural Selection. Oxford Univ. Press, 1999.
6. *Bürger R.* A multilocus analysis of intraspecific competition and stabilizing selection on a quantitative trait // J. Math. Biol. 2005. V.50. №4. P. 355–396.
7. *Bürger R., Bagheri H.C.* Dominance and Its Evolution. In: Jorgensen S.E.; Fath B. Encyclopedia of Ecology. Amsterdam, Elsevier, 2008, P.945-952.
8. *Bagheri H.C.* Unresolved boundaries of evolutionary theory and the question of how inheritance systems evolve: 75 years of debate on the evolution of dominance // J. Exp. Zool. B Mol. Dev. Evol. 2006. V.306. №4. P. 329-359.
9. *Kopp M., Hermisson J.* The evolution of genetic architecture under frequency-dependent disruptive selection // Evolution. 2006. V.60. №8. P.1537-1550.
10. *Johnson T., Barton N.* Theoretical models of selection and mutation on quantitative traits // Philos. Trans. R. Soc. Lond. B Biol. Sci. 2005. V.360. №1459. P.1411–1425.
11. *Bagheri H.C., Wagner G.P.* Evolution of dominance in metabolic pathways // Genetics. 2004. V.168. №3. P. 1713-1735.
12. *Haldane J.B.S.* A note on Fisher's theory of the origin of dominance, and on a correlation between dominance and linkage // Am. Nat. 1930. V.64. №690. P. 87-90.
13. *Haldane J.B.S.* The theory of the evolution of dominance // J. Genet. 1939. V.37. №2. P. 365-374.
14. *Kulikov A.M., Melnikov A.I., Gornostaev N.G., Lazebny O.E., Mitrofanov V.G.* Morphological analysis of male mating organ in the *Drosophila virilis* species group: a multivariate approach // J. Zool. Syst. Evol. Research. 2004. V. 42. № 2. P. 135-144.
15. Kulikov A.M., Mel'nikov A.I., Gornostaev N.G., Lazebny O.E. Dominance status of shape of male genitalia in interspecific crosses of some Drosophila virilis group species. Russian Journal of Genetics. 2013. V. 49. № 6. P. 588-601.
16. *Eberhard W.G.* Sexual selection and animal genitalia. Cambridge, MA: Harvard University Press, 1985, 244 pp.

17. Bei-Bienko G.Ya. Part 1. Hard-winged and fan-winged / / Determinant of insects in the European part of the USSR in five volumes. - Moscow-Leningrad: "Science", 1965. - T. II. - 668
18. *Spirina T.S.* Comparative morphology of male and female genitalia of two forms of four-spotted seed *Callosobruchus macullatus* // Entomologicheskoe Obozrenie. 1974. V. 3. N. 4. P. 752-760 (in Russian)
19. *Kasatkin D.G.* Endopallus of beetle beetles (Coleoptera, Cerambycidae) and its significance in the resolution of taxonomic problems. Thesis for the degree of candidate of biological sciences by specialty 03.00.09 "Entomology", 2003, Moscow, 209 p.
20. *Ler P.A.* Part 1. Caddis flies and Lepidoptera // The determinant of insects of the Russian Far East in five volumes, - Vladivostok: "Dalnauka", 1997. - T. V. - 540 p.
21. *Ler P.A.* Part 2. Caddis flies and Lepidoptera // The determinant of insects of the Russian Far East in five volumes, - Vladivostok: "Dalnauka", 1997. - T. V. - 671 p.
22. *Kuznetsov V.I.* New approaches to the system of Lepidoptera of the world fauna (based on the functional morphology of the abdomen) / V.I. Kuznetsov, A.A. Stekolnikov. SPb .: Science, 2001, 462 p.
23. *Belyaev E.A.* Phylogenetic connections of the family of moths and its subfamilies (Lepidoptera: Geometridae) // Readings of NA Kholodkovskii's memory. - Vol. 60.- St. Petersburg: Zool. Institute of the Russian Academy of Sciences, 2008, 238 p.
24. *Lyubishchev A.A.* Problems of the form of the systematics of the evolution of organisms. Science, Moscow, 1982, 278 p.
25. *Jagadeeshan S., Singh R.S.* A time-sequence functional analysis of mating behaviour and genital coupling in Drosophila: role of cryptic female choice and male sex-drive in the evolution of male genitalia // J. Evol. Biol. 2006. V. 19. №4. P. 1058-1070.
26. *Mazzi D., Kesaniemi J., Hoikkala A., Klappert K.* Sexual conflict over the duration of copulation in *Drosophila montana*: why is longer better? // BMC Evol. Biol. 2009. V. 9. P. 132.
27. *Kulikov A.M., Gornostaev N.G., Lazebny O.E.* Interspecies variability of number of bristles on dorsal surface of aedeagus in D. virilis species group and its genetic mapping with interspecies hybrids of D. virilis and D. lummei. Russian Journal of Genetics. 2013. V. 49. № 2. P. 158-163.
28. *Johnson T., Barton N.* Theoretical models of selection and mutation on quantitative traits // Philos. Trans. R. Soc. Lond. B Biol. Sci. 2005. V .360. №1459. P. 1411–1425.
29. *Zhang X.-S., Hill W.G.* Predictions of Patterns of Response to Artificial Selection in Lines Derived From Natural Populations // Genetics. 2005. V. 169. №1. P. 411-425.

30. *Carson H.L., Lande R.* Inheritance of a secondary sexual character in *Drosophila silvestris* // Proc. Natl. Acad. Sci. USA. 1984. V. 81. №21. P. 6904-6907.
31. *Orr H.A., Betancourt A.J.* Haldane's sieve and adaptation from the standing genetic variation // Genetics. 2001. V. 157. №2. P.875-884.
32. *Wayne M.L., Pan Y.J., Nuzhdin S.V., McIntyre L.M.* Additivity and trans-acting effects on gene expression in male *Drosophila simulans*. Genetics. 2004. V.168, №3, P.1413-1420.
33. *Wang H.Y., Fu Y., McPeek M.S., Lu X., Nuzhdin S., Xu A., Lu J., Wu M.L., Wu C.I.* Complex genetic interactions underlying expression differences between Drosophila races: analysis of chromosome substitutions // Proc. Natl. Acad. Sci. USA. 2008. V. 105. №17. P. 6362-6367.
34. *Kotelnikov R.N., Klenov M.S., Rozovsky Y.M., Olenina L.V., Kibanov M.V., Gvozdev V.A.* Peculiarities of piRNA-mediated post-transcriptional silencing of Stellate repeats in testes of *Drosophila melanogaster* // Nucleic Acids Res. 2009. V.37. №10. P 3254--3263.
35. *Olenkina O.M., Egorova K.S., Kibanov M.V., Gervaziev Y.V., Gvozdev V.A., Olenina L.V.* Promoter contribution to the testis-specific expression of Stellate gene family in *Drosophila melanogaster* // Gene. 2012. V. 499. №1. P.143--153.
36. *Bayes J.J., Malik H.S.* Altered heterochromatin binding by a hybrid sterility protein in Drosophila sibling species // Science. 2009. V. 326. №5959. P. 1538-1541.
37. *Lu X., Shapiro J.A., Ting C.T., Li Y., Li C., Xu J., Huang H., Cheng Y.J., Greenberg A.J., Li S.H., Wu M.L., Shen Y., Wu C.I.* Genome-wide misexpression of X-linked versus autosomal genes associated with hybrid male sterility // Genome. Res. 2010. V. 20. №8. P.1097-1102.
38. *Päällysaho S., Aspi J., Liimatainen J.O., Hoikkala A.* Role of X Chromosomal Song Genes in the Evolution of Species-Specific Courtship Songs in Drosophila virilis Group Species. Behav Genet. 2003 Jan; V.33, №1, P.25-32.
39. *Carvalho A.B., Dobo B.A., Vibranovski M.D., Clark A.G.* Identification of five new genes on the Y chromosome of *Drosophila melanogaster* // Proc. Natl. Acad. Sci. USA. 2001. 6; V. 98, №23, P. 13225-13230.
40. *Piergentili R., Mencarelli C. Drosophila melanogaster* kl-3 and kl-5 Y-loops harbor triple-stranded nucleic acids // J. Cell. Sci. 2008. V. 121. Pt. 10. P. 605-612.
41. *Vibranovski M.D., Koerich L.B., Carvalho A.B.* Two new Y-linked genes in *Drosophila melanogaster* // Genetics. 2008. V. 179. №4. P. 2325-2327.
42. FLIGHT Database
43. *Menon D.U., Meller V.H.* Imprinting of the Y chromosome influences dosage compensation in roX1 roX2 *Drosophila melanogaster* // Genetics. 2009. V. 183. № 3. P. 811-820.
44. *Deng X., Koya S.K., Kong Y., Meller V.H.* Coordinated regulation of heterochromatic genes in *Drosophila melanogaster* males // Genetics. 2009. V. 182. №2. P. 481-491.

45. *Lemos B., Araripe L.O., Hartl D.L.* Polymorphic Y chromosomes harbor cryptic variation with manifold functional consequences // Science. 2008. V.319. №5859, P.91-93.

Table 1. MPs and corresponding landmarks

MP	Pairs of points (Fig. 1)	Description
1	1–2	Aedeagus length to the cook base
2	4–5	Maximum aedeagus length
3	2–3	Cook length
4	6–8	Distance from the MP1 axis to the lower outline as measured 93.75% of the MP1 length away from point 1
5	7–8	Aedeagus height measured 93.75% of the MP1 length away from point 1
6	9–11	Distance from the MP1 axis to the lower outline as measured 75% of the MP1 length away from point 1
7	10–11	Aedeagus height measured 75% of the MP1 length away from point 1
8	12–14	Distance from the MP1 axis to the lower outline as measured 50% of the MP1 length away from point 1
9	13–14	Aedeagus height measured 50% of the MP1 length away from point 1
10	16–17	Aedeagus height measured 25% of the MP1 length away from point 1
11	15–17	Distance from the MP1 axis to the lower outline as measured 25% of the MP1 length away from point 1
12	1–18	Aedeagus height at the base
13	19–20	Maximum distance from the MP1 axis to the upper outline of the projection
14	20–1	Distance from the aedeagus base to the projection of the highest point of the outline onto the MP1 axis
15	21–22	Distance from the MP1 axis to the inflection point of the upper outline
16	22–1	Distance from point 1 to the projection of the inflection point of the upper outline onto the MP1 axis
17	23–24	Distance from point 1 to the projection of the lowest point of the aedeagus outline onto the MP1 axis
18	23–1	Distance from the MP1 axis to the lowest point of the aedeagus outline
19	25–1	Distance from the projection of the uppermost point of the ventral aedeagus on the MP1 axis to point 1
20	26–25	Distance from the MP1 axis to the upmost point of the ventral part of the aedeagus outline
21	27–1	Gonite width at the base
22	32–34	Curve depth of the ventral part of the gonite
23	27–28	Distance from the MP1 axis to the gonite outline as measured at the base
24	28–35	Gonite length at the ventral curvature
25	29–30	Distance from the MP23 midpoint to the outline of the basal part of the

		gonite (characterizes the gonite bending angle)
26	30–31	Gonite length in the central part (at the MP23 midpoint)
27	33–34	Gonite height in the central part (at the MP26 midpoint)
28	1–36	Apodeme length
29	38–39	Apodeme width measured 25% of the MP28 length away from point 1
30	37–38	Apodeme bending parameter (25% of the MP28 length away from point 1)
31	41–42	Apodeme width measured 50% of the MP28 length away from point 1
32	40–41	Apodeme bending parameter (50% of the MP28 length away from point 1)
33	44–45	Apodeme width measured 75% of the MP28 length away from point 1
34	43–44	Apodeme bending parameter (75% of the MP28 length away from point 1)

Table 2. Trait dominance in the shape of the copulatory system in *D. virilis/D. lummei* hybrid males differing in the origin of the sex chromosomes

Factor / MPI		♂$_{Vi}$ x ♀$_{Lu}$ F_1 $X_{Lu}Y_{Vi}$		♂$_{Lu}$ x ♀$_{Vi}$ F_1 $X_{Vi}Y_{Lu}$		♂F_1(♂$_{Lu}$ x ♀$_{Vi}$) x ♀$_{Vi}$ F_b $X_{Vi}Y_{Lu}$		♂F_1(♂$_{Vi}$ x ♀$_{Lu}$) x ♀$_{Vi}$ F_b $X_{Vi}Y_{Vi}$	
		D_x	P.-h.	D_x	P.-h.	D_x	P.-h.	D_x	P.-h.
F1	30	D_{Lu}	f_1,l<v	D_{Vi}	l<f_b,v	D_{Lu}	l,f_b<v	ID	l≤f_b≤v
	32	N	f_1<l,v	ns	f_1,l,v	N	f_b≤l,v	ns	l,f_b,v
	34	oD_{Vi}	f_1<v≤l	ns	v,f_1,l	D_{Vi}	f_b,v<l	ns	f_b,v,l
	β	D_{Lu}	l,f_1<v	D_{Vi}	l<v,f_1	D_{Lu}	l,f_b<v	D_{Vi}	l<f_b,v
F2	6	D_{Vi}	f_1,v<l	ID	f_1≤v≤l	D_{Vi}	f_b,v<l	ID	v≤f_b≤l
	8	ID	v≤f_1≤l	D_{Lu}	v<f_1,l	D_{Vi}	v,f_b<l	D_{Vi}	v,f_b<l
	17	D_{Vi}	f_1,v<l	ID	v<f_1≤l	D_{Vi}	f_b,v<l	D_{Vi}	v,f_b<l
	18	ID	l<f_1<v	D_{Lu}	f_1,l <v	ID	l<f_b<v	D_{Vi}	l<f_b,v
	19	D_{Vi}	l<f_1,v	ID	l<f_1<v	ID	l≤f_b≤v	D_{Lu}	l,f_b≤v
F3	12	ID	v≤f_1≤l	ID	v≤f_1≤l	ID	v≤f_b≤l	D_{Vi}	f_b,v<l
	33	D_{Vi}	l<v,f_1	ns	l,f_1,v	ns	f_b,v,l	D_{Lu}	l,f_b<v
F4	10	N	v,l<f_1	ns	v,f_1,l	ns	v,f_b,l	ns	v,f_b,l
	20	D_{Lu}	v<f_1,l	D_{Vi}	v,f_1<l	D_{Vi}	v,f_b<l	ID	v≤f_b≤l
F5	24	D_{Vi}	l<f_1,v	D_{Vi}	l<f_1,v	D_{Lu}	l,f_b<v	ID	l,f_b<v
F6	4	ns	f_1,l,v	ns	f_1,l,v	N	f_b<l,v	ns	f_b,l,v
F7	2	oD_{Lu}	f_1<l≤v	ns	f_1,l≤v	ns	f_b,l≤v	ns	l,f_b,v
	α	oD_{Vi}	l<v<f_1	oD_{Vi}	l<v<f_1	D_{Vi}	l<f_b,v	ID	l<f_b<v
F8	5	D_{Lu}	f_1,l<v	D_{Lu}	f_1,l<v	D_{Lu}	f_b,l<v	D_{Lu}	l,f_b<v
	7	D_{Lu}	f_1,l<v	D_{Lu}	l,f_1<v	D_{Lu}	f_b,l<v	D_{Lu}	l,f_b<v
	14	ID	l<f_1<v	ID	l<f_1<v	ID	l<f_b<v	D_{Lu}	l,f_b<v
	16	ID	l<f_1<v	ID	l<f_1<v	ID	l<f_b<v	ID	l≤f_b<v
	26	ID	l<f_1<v	ID	l<f_1<v	ID	l<f_b<v	ID	l<f_b<v
F8; 9	11	ID	v<f_1<l	D_{Lu}	v<l,f_1	ID	v<f_b<l	D_{Vi}	v,f_b<l
F9 9	9	D_{Vi}	l<v,f_1	oD_{Vi}	l<v<f_1	D_{Vi}	l<v,f_b	D_{Vi}	l<v,f_b
	13	ns	l,v,f_1	ns	l,v,f_1	ns	f_b,l,v	ns	f_b,l,v
	15	D_{Vi}	l<v,f_1	D_{Vi}	l<v,f_1	D_{Vi}	l<v,f_b	ns	l,f_b,v
	28	D_{Lu}	f_1,l<v	ns	l,f_1,v	D_{Lu}	l,f_b<v	ns	l,f_b≤v

Traits are grouped according to their maximal weights in the factors isolated by a principal component analysis (Table 5). MPs 3, 21, 22, 23, 25, 27, 29, and 31 did not show a significant effect of the genotype on trait variation and are omitted. D_x, dominance: D_{Lu}, dominance of the *D. lummei* phenotype; D_{Vi}, dominance of the *D. virilis* phenotype; oD_{Lu} and oD_{Vi}, overdominance of the *D. lummei* or *D. virilis* phenotype, respectively; ID, intermediate dominance; N, a new phenotype; ns, difference is nonsignificant. Crosses and paternal genotypes are shown in the upper row. P.-h., results of post hoc comparisons: f_1, f_b, l, and v are the mean trait values in F_1, backcross, *D. lummei*, and *D. virilis* males, respectively; symbols are shown in

the order of increasing trait value. Genotypes combined in one group homogeneous in terms of trait variation are separated by commas.

Table 3. Effects of the sex chromosomes and parental genotypes on trait expression

	X		Y		X*Y		♂P		♂P*X	
	F	p H-B	F	p H-B	F	p H-B	F	p H-B	F	p H-B
mp2	19.56	0.00		ns		ns	19.02	0.00		ns
mp8		ns		ns		ns	7.82	0.02		ns
mp10	13.65	0.01		ns		ns	31.40	0.00	48.06	0.00
mp11		ns	11.00	0.04		ns	13.17	0.00	20.38	0.00
mp13	15.61	0.00		ns		ns	12.88	0.00		ns
mp17		ns		ns		ns	10.47	0.00		ns
mp18		ns	13.22	0.01	12.43	0.02		ns	10.82	0.04
mp19		ns		ns		ns	6.39	0.05	15.59	0.00
mp20		ns		ns		ns	26.01	0.00	43.95	0.00
mp26		ns		ns		ns	6.84	0.04		ns
mp30		ns		ns		ns	19.90	0.00	21.48	0.00
mp32		ns		ns		ns	20.33	0.00	22.64	0.00
mp33		ns		ns		ns	7.57	0.02		ns
mp34	12.31	0.02		ns		ns	15.41	0.00	15.54	0.00
alpha	32.89	0.00	18.05	0.00	24.12	0.00	40.51	0.00		ns
beta		ns		ns		ns	8.21	0.01		ns

F, Fisher's F statistic; p H-B, the probability of accepting the null hypothesis, with Holm–Bonferroni correction for multiple testing. Estimates were obtained for the factors factors X chromosome, Y chromosome, and paternal genotype (♂P) and their pairwise combinations. The combination Y chromosome–paternal genotype did not show significant deviations from the null hypothesis.

Table 4. Factor loadings obtained by the principal component analysis and their dependence on the progeny genotype

Factor 1 (8.09; 0.000)	Factor 2 (6.66; 0.000)	Factor 3 (1.39; 0.23)	Factor 4 (11.13; 0.000)	Factor 5 (1.23; 0.30)
30*, 32*, 34*, β	6*, 8*, 17*, 18, 19	12, 29, 31*, 33*	10*, 20*	22*, 24, 25
Factor 6 (7.85; 0.000)	**Factor 7 (33.34; 0.000)**	**Factor 8 (32.26; 0.000)**	**Factor 9 (6.15; 0.000)**	**Factor 10 (2.55; 0.092)**
4, 21	2*, α*	5, 7*, 11, 14*, 16*, 26	9, 11, 13*, 15*	23*, 27*

Traits included in factor loadings with weights higher than 0.5 are shown; traits that have weights higher than 0.7 are indicated with (*).

* - traits that have weights higher than 0.7.

Fisher's F statistic and the probability of accepting the null hypothesis, with Holm–Bonferroni correction for multiple testing, are given in parentheses.

Figure Captions

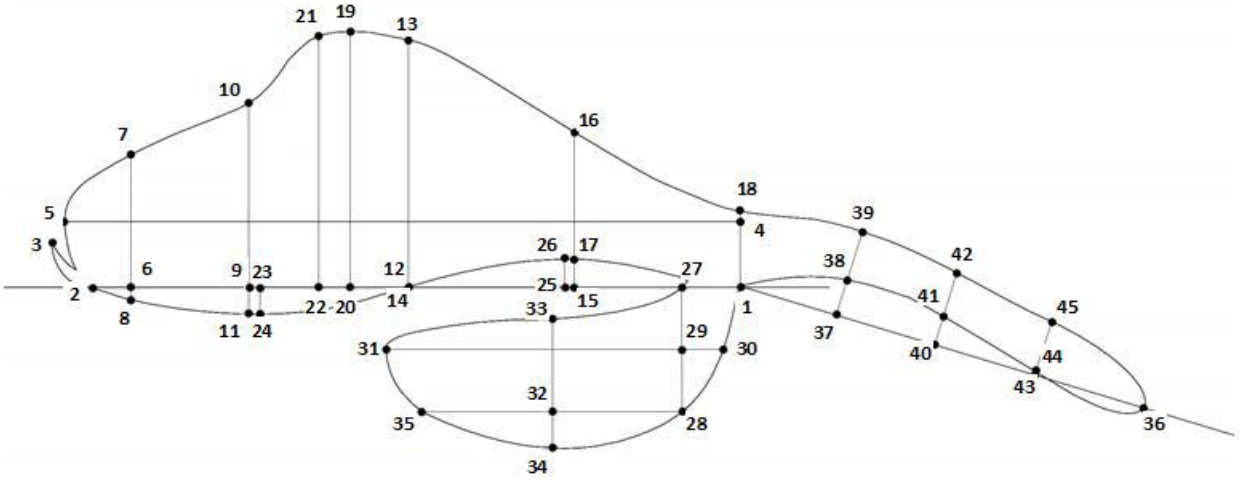
Fig. 1. Scheme of a coordinate grid and points marked on the outline of the lateral view of the *Drosophila* copulatory organ.

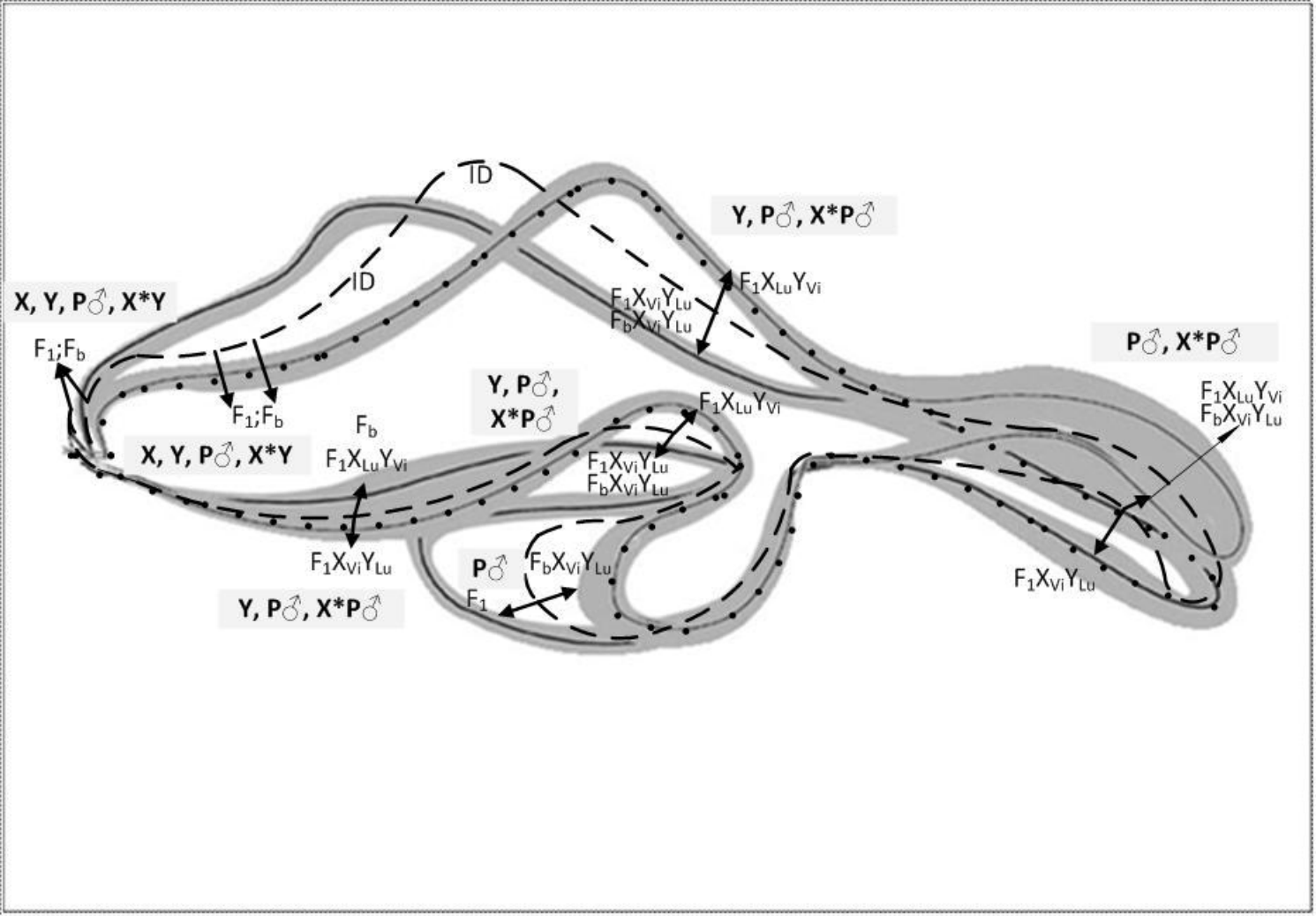
MPs and corresponding points are summarized in Table 1.

Fig. 2. Scheme of trait dominance in the shape of the copulatory organ in interspecific hybrids.

The outline of the copulatory organ is shown with a solid line for *D. virilis*, a solid line with dots for *D. lummei*, and a dashed line for the interspecific hybrids (an averaged outline). Regions covering 95% of the trait variation in the parental species are shown gray. Changes in traits towards dominance of one of the parental genotypes are indicated with arrows starting from the dashed outline. F_1 or F_b indicates that the two genotypes showed similar dominance in F_1 from reciprocal crosses or backcrosses, respectively. When both of the genotypes showed similar dominance in the reciprocal cross or backcross progenies, designations are given accordingly. Intermediate dominance estimates are shown only for the evolutionarily contrast traits of factor 8 and are the same for all genotypes.

The sex chromosomes and the paternal genotype P♂ as an epigenetic factor are shown in frames for the copulatory system regions where they significantly affected trait dominance in the heterozygous genotypes.


21
19
13
10
16
7
18
5
39
4
3
26
17
42
6
9
23
12
27
38
2
25
41
14
15
1
45
8
22
20
33
37
11
24
29
40
31
30
44
32
43
35
28
36
34


TD
ID
X, Y, P♂, X*Y
F1;Fb
F1;Fb
X, Y, P♂, X*Y
Fb
F1XLuYVi
F1XViYLu
Y, P♂, X*P♂
Y, P♂,
X*P♂
F1XLuYVi
F1XVi YLu
FbXVi YLu
P♂
F1
FbXViYLu
Y, P♂, X*P♂
F1XLuYVi
F1XVi YLu
FbXVi YLu
P♂, X*P♂
F1XLuYVi
FbXViYLu
F1XViYLu