A balanced inversion polymorphism exhibits a dominance reversal at the gene expression level that depends on developmental context

Esra Durmaz Mitchell[1,2,*], Envel Kerdaffrec[1,*], Paul Schmidt[3], Thomas Flatt[1,†,‡] and Sebastian Kittelmann[4,†,‡]

Author Affiliations:

[1]Department of Biology, University of Fribourg, Fribourg, Switzerland

[2]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

[3]Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania, USA

[4] Centre for Functional Genomics, School of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom

*equal contribution

[†]shared last authorship

[‡] thomas.flatt@unifr.ch; skittelmann@brookes.ac.uk

ORCID IDs:

EDM: 0000-0002-4345-2264

EK: 0000-0001-8667-6850

PS: 0000-0002-8076-6705

TF: 0000-0002-5990-1503

SK: 0000-0002-2234-4539

Short Running Title: Dominance reversal of inversion karyotypes

Abstract

How genetic variance for fitness is maintained is incompletely understood. Mutation-selection balance and single-locus overdominance cannot account for the large variance observed. Recent work suggests that antagonistic balancing selection, favoring different alleles in different contexts and involving beneficial dominance reversals, might contribute to maintaining fitness variance. However, while this mechanism is plausible, evidence for dominance reversals remains scarce. Here, we study how *In(3R)Payne*, a balanced inversion polymorphism in *D. melanogaster*, affects gene expression and chromatin accessibility by using RNA-seq and ATAC-seq. We find that, in embryos, the inverted (INV) arrangement tends to have dominant effects, while the standard (STD) arrangement behaves like a recessive Mendelian allele. Yet, in wing discs, this pattern is reversed: STD has mostly dominant effects, whereas INV behaves recessively. Since this shift in the dominance of the INV "allele" between developmental contexts affects the expression of suites of genes in a concerted manner, it might be mediated by a dominance modifier, for example a transcription factor. In favor of this idea, 25% of the differentially expressed genes between INV and STD encode transcription factors. Interestingly, while only four differentially expressed genes are shared between embryos and wing discs, one of them is *HP1c*, a chromatin binding protein and major transcriptional regulator, and thus a promising candidate for mediating the context-dependent change in dominance. Although the relationship between these patterns and fitness is presently unknown, our observations are consistent with a potential role of reversals (or, more generally, shifts) of dominance in maintaining inversion polymorphism.

Keywords: balancing selection, dominance reversal, fitness variance, inversion polymorphism, gene expression, chromatin accessibility.

Introduction

A longstanding fundamental paradox in population genetics is the observation that fitness-related traits (i.e., those subject to strongest selection) typically harbor substantial amounts of genetic variation despite natural selection relentlessly eroding this variation by fixing beneficial variants and removing deleterious ones (Dempster 1955; Lewontin 1974; Lande 1975; Barton & Turelli 1989; Lynch & Walsh 1998; Houle 1992; Charlesworth & Hughes 2000; Barton & Keightley 2002; Turelli and Barton 2004; Johnson & Barton 2005; Walsh & Blows 2009; Charlesworth 2015; Charlesworth & Charlesworth 2017; Connallon & Chenoweth 2019; Flatt 2020; Grieshop et al. 2024).

On the one hand, too much variance seems to exist for fitness components to be accounted for solely by mutation-selection balance (e.g., Charlesworth & Hughes 2000; Charlesworth 2015; also see Sharp & Agrawal 2018). On the other hand, balancing selection in the form of classical single-locus overdominance seems to be rare (e.g., Parsons & Bodmer 1961; Gemmell & Slate 2006; Andrés et al. 2009; Hedrick 2011, 2012; Fijarczyk & Babik 2015; Croze et al. 2016, 2017; Stephan & Hörger 2019), likely due to segregation load (Crow 1970; Dolgin & Otto 2003), and can therefore not account for the high amount of fitness variance observed in natural populations (Charlesworth & Hughes 2000; Charlesworth 2015).

These findings suggest that some types of balancing selection other than classical single-locus overdominance might be involved in maintaining genetic variance in fitness (Charlesworth & Hughes 2000; Charlesworth 2015). Indeed, there is a large variety of mechanisms of balancing selection such as negative frequency-dependent selection, multi-locus heterozygote advantage including epistatic balancing selection, genotype by environment interactions, as well as different kinds of antagonistic selection which can generate balancing selection (e.g., antagonistic pleiotropy, sexually antagonistic selection, and spatially or temporally varying selection); importantly, several of these mechanisms can lead to so-called marginal overdominance or net heterozygote advantage for fitness (e.g., Fisher 1930a; Haldane 1948; Levene 1953; Dempster 1955; Lewontin 1958; Haldane & Jayakar 1963; Gillespie & Langley 1974; Ayala & Campbell 1974; Hedrick 1976; Gillespie 1978; Rose 1982, 1985; Curtsinger et al. 1994; Charlesworth & Hughes 2000; Prout 2000; Turelli & Barton 2004; Van Dooren 2006; Patten et al. 2010; Connallon & Clark 2012, 2013, 2014; Barson et al. 2015; Spencer and Priest 2016; Brisson 2018; Brown & Kelly 2018; Grieshop & Arnqvist 2018; Zajitschek & Connallon 2018; Connallon & Chenoweth 2019; Xu et al. 2019; Bitarello et al. 2023; Berdan et al. 2023; Christie & McNickle 2023; Grieshop et al. 2024; Siljestam et al. 2024; Flintham et al. 2025; Khudiakova et al. 2025; Guyot et al. 2025; Ruzicka et al. 2025). In terms of evolutionary timescales, these mechanisms can either

lead to recent balancing selection, which may or, more commonly, may not maintain a balanced polymorphism in the long run, or to long-term balancing selection, which maintains a balanced polymorphism for a timescale longer than the neutral timescale (Bitarello et al. 2023).

In view of the inexplicably high variance for fitness and the apparent scarcity of classical single-locus overdominance, "non-classical" forms of balancing selection have recently (re-) gained considerable interest (e.g., see Johnston et al. 2013; Connallon & Clark 2013; Bergland et al. 2014; Wittmann et al. 2017; Grieshop & Arnqvist 2018; Bertram & Masel 2019; Connallon & Chenoweth 2019; Machado et al. 2021; Glaser-Schmitt et al. 2021; Chevin et al. 2022; Rudman et al. 2022; Hoffmann & Flatt 2022; Rusuwa et al. 2022; Bitarello et al. 2023; Berdan et al. 2023; Kapun et al. 2023; Arnqvist & Rowe 2023; Pei et al. 2023; Wittmann et al. 2023; Johnson et al. 2023; Kim 2023; Grieshop et al. 2024; Siljestam et al. 2024; Flintham et al. 2025; Brud 2025). This is especially true for antagonistic balancing selection, i.e., the phenomenon whereby alternative alleles are favored in different contexts (e.g., different habitats, niches, seasons, sexes, or fitness traits), including beneficial reversals of dominance of alternative alleles (e.g., see Connallon & Clark 2013; Connallon & Chenoweth 2019; Grieshop et al. 2024, and references therein).

Beneficial dominance reversals imply that a given allele is partially or fully dominant in the context in which it is selectively favored but recessive in the alternative context in which it is deleterious (e.g., Kidwell et al. 1977; Rose 1982; Curtsinger et al. 1994; Wittmann et al. 2017; Connallon & Chenoweth 2019; Grieshop et al. 2024). Such dominance reversals can lead to an average net advantage of the heterozygote across different contexts (marginal overdominance; see above) and thus contribute to maintaining variation (e.g., Wallace 1968; Gillespie & Langley 1974; Kidwell et al. 1977; Rose 1982, 1985; Curtsinger et al. 1994; Charlesworth & Hughes 2000; Posavi et al. 2014; Connallon & Chenoweth 2019; Grieshop et al. 2024; Karageorgi et al. 2024). While recent modeling suggests that the conditions whereby (sexually) antagonistic balancing selection and dominance reversals can maintain long-term polymorphism at loci underlying continuous traits might be quite restrictive unless large-effect loci are involved and/or linkage between loci is tight (Flintham et al. 2025), other approaches indicate that beneficial dominance modifications might evolve quite readily and can promote polymorphism even under fairly weak selection (Siljestam et al. 2024; also cf. Wittmann et al. 2017). Moreover, new theoretical work by Brud (2025) has found that, beyond beneficial dominance reversals, also context-dependent (e.g., seasonal), *non-reversing* changes in dominance can stabilize biallelic polymorphism. Yet, despite the theoretical plausibility of such context-dependent reversals (or, more generally, shifts) of

dominance, empirical evidence for their existence and involvement in maintaining polymorphisms remains relatively scarce (e.g., Johnston et al. 2013; Posavi et al. 2014; Grieshop & Arnqvist 2018; Kaufmann et al. 2024; Karageorgi et al. 2024; reviewed in Connallon & Chenoweth 2019 and Grieshop et al. 2024).

Here we report a new example of a dominance reversal involving an adaptive inversion polymorphism in *Drosophila melanogaster*, *In(3R)Payne* (or *3RP*), an approximately 8-Mb large paracentric chromosomal inversion that encompasses ~1,200 genes on chromosome arm *3R*. This common, cosmopolitan polymorphism consists of two alternative chromosomal arrangements, a non-inverted (standard = STD) arrangement and an inverted (=INV) arrangement, and seems to be maintained by multiple forms of balancing selection, including evidence for spatially varying selection, temporally varying selection, negative-frequency dependent selection as well as overdominant selection (Nassar et al. 1973; Lemeunier & Aulard 1992; Kapun et al. 2016a; Kapun & Flatt 2019; Kapun et al. 2023; Paris et al. 2025). Our recent population genomic analyses suggest that this polymorphism likely represents a long-term balanced polymorphism (Kapun et al. 2023). Previous work has found that *3RP* affects a whole suite of fitness components, including various developmental and size-related traits, pigmentation, fecundity, several stress resistance traits as well as lifespan (Rako et al. 2006; Kennington et al. 2007; Kapun et al. 2016b; Durmaz et al. 2018; Kapun & Flatt 2019; Paris et al. 2025). The *3RP* polymorphism thus seems to represent a supergene, a set of tightly linked loci which jointly affect several complex phenotypes (Thompson & Jiggins 2014; Schwander et al. 2014; Gutiérrez-Valencia et al. 2021; Berdan et al. 2022; Schaal et al. 2022). Yet, the functional genetic and molecular architecture of the *3RP* inversion and the selective mechanisms that maintain it as a balanced polymorphism remain incompletely understood (cf. discussion in Kapun et al. 2023 and Paris et al. 2025).

A promising approach for identifying functionally relevant genes and variants associated with inversion polymorphism is expression profiling (Fuller et al. 2016; Lavington & Kern 2017; Said et al. 2018; Giner-Delgado et al. 2019; Crow et al. 2020; Berdan et al. 2021; Kapun et al. 2023). To learn how the *3RP* inversion polymorphism affects gene expression and gene regulation during development we performed RNA-seq expression profiling of embryos and wing discs (see Kapun et al. 2023 for data on adult expression) as well as ATAC-seq analysis (Buenrostro et al. 2013, 2015) of wing discs.

To “capture” some of the allelic variation segregating among different chromosomes within the STD karyotype and within the INV karyotype, we chose to perform our assays using two genetic backgrounds, namely two independent STD chromosomes and two independent INV

chromosomes, and their respective INV/STD heterokaryotypes (HET), resulting in two distinct "replicate" sets of INV/INV, STD/STD and HET karyotypes. This experimental design allowed us to make an inference about the "average" behavior of the different *3RP* karyotypes instead of only comparing the three karyotypes based on a single INV vs. a single STD chromosome, even though – admittedly – this level of biological replication is minimal.

By comparing expression between STD/STD, INV/INV and HET, and by quantifying dominance coefficients for genes that are differentially expressed between these karyotypes, we discover that, in HET embryos, the INV chromosomal arrangement tends (on average across the two genetic backgrounds; see above) to be dominant in affecting the expression of most genes, while the STD arrangement appears to have mostly recessive effects. In wing discs, by contrast, this pattern is reversed, with the STD arrangement having mostly dominant effects, while the INV arrangement tends to be recessive. Using ATAC-seq analysis of wing discs, we further find that the dominance patterns in terms of differential expression are qualitatively mirrored by matching patterns of chromatin accessibility. The two alternative chromosomal arrangements thus seem to behave like Mendelian alleles and exert coordinated, "switch-like" effects on the expression and chromatin accessibility of numerous genes spanned by the inversion, consistent with the notion of a supergene. Finally, we identify a major transcriptional regulator, the chromatin binding protein *HP1c*, as a candidate "dominance modifier" (Fisher 1928, 1930a,b; Sved & Mayo 1970; Ohh & Sheldon 1970; Thomson & Thoday 1972; McKenzie & Game 1987; Doebley et al. 1995; Davies et al. 1996; Otto & Bourguet 1999; Tarutani et al. 2010; Billiard & Castric 2011; Di & Lohmueller 2024) which might mediate the observed dominance reversal between embryos and wing discs.

Our study thus provides a "proof of principle" for the involvement of reversals (or, more generally, shifts) of dominance in inversion-specific gene expression and, potentially, in maintaining balanced inversion polymorphism (also see Pearse et al. 2019; Mérot et al. 2020), albeit the fitness relevance of the observed patterns remains unknown at present (but cf. Paris et al. 2025).

Results and Discussion

In embryos, the INV arrangement has a dominant effect on expression

To understand how the *3RP* inversion polymorphism affects global transcriptional output during development, we first performed gene expression analysis during embryogenesis at 4-6 h after egg laying. This stage of development lays the foundation for many phenotypic features of the adult organism, as several important developmental genes become

expressed for the first time. This includes genes that are spanned by *3RP*, e.g., the homeotic selector genes. To determine expression changes that are due to the INV vs. the STD karyotype we generated lines that differ in *3RP* karyotype but which otherwise had the same genetic background (see Materials and Methods). To account for allelic differences among different INV haplotypes and among different STD haplotypes, we combined and contrasted data from two different INV vs. those from two different STD genotypes (see above; see Materials & Methods; for individual line comparisons see Fig. S1 and Fig. S2).

We first compared differential expression between INV and STD homokaryotypes and identified 125 differentially expressed genes (DEG; adjusted *p* [p_{adj}] ≤ 0.001; Fig. 1A, Table S1), with more than a third of them being located on *3R* and 14.4% within the region spanned by *3RP* (Fig. 1B, Table S1). In total, 1.14% of all genes on *3R* were differentially expressed, and 1.47% of the genes in the region spanned by *3RP*. In contrast, the fraction of DEG on other chromosome arms (*X*, *2L*, *2R*, *3L*, and other [comprising *4*, *Y*, and unmapped scaffolds]) ranged between 0.38 and 0.99% (Fig. 1G, Table S1). Thus, while a significant portion of differential expression between INV and STD occurs on *3R* and in the region spanned by *3RP*, the inversion polymorphism clearly has genome-wide effects on expression, as previously observed for several polymorphic *D. melanogaster* inversions including *3RP* and consistent with *trans*-acting regulatory effects (Lavington & Kern 2017; Said et al. 2018; Kapun et al. 2023; also see below).

Next, we compared the expression profiles of HET flies to those of the parental INV/INV and STD/STD homokaryotypes in a pairwise manner. Only four genes showed expression differences between HET and the INV homokaryotype, while 176 genes were differentially expressed between HET and STD (p_{adj} ≤ 0.001; Fig. 1C-F, Table S1). As our experimental design has a relatively low degree of replication, it likely underestimates the true number of DEGs. However, even with a much less stringent p_{adj}-value cut-off of 0.1, only seven genes were differentially expressed between HET and INV, while the number of DEG between HET and STD rose to 924 and between STD and INV to 873 (for detailed quantification and proportional values see Table S1). Indeed, a large fraction of the DEG in the HET vs. STD comparison was shared with the STD vs. INV comparison (85 genes at p_{adj} ≤ 0.001; 626 genes at p_{adj} ≤ 0.1; Fig. 1I, Table S1). All 4 DEG in the HET vs. INV comparison were also found in STD vs. INV, as well as 6 genes at p_{adj} ≤ 0.1 (Fig. 1I). At this cut-off, 2 genes were shared between all comparisons. The similarity of the HET and INV datasets is also apparent when assessing Spearman's rank correlations of normalized read counts between the different datasets (Fig. S1G).

Interestingly, these pairwise comparisons of the three karyotypes suggest a pattern that is reminiscent of a dominant mode of Mendelian inheritance whereby INV/INV ≈ HET > STD/STD, i.e., the INV "allele" is dominant over the STD "allele" (compare the expression patterns in Fig. 1A and 1C with that in 1E; also see below).

About one quarter of DEG between HET and STD were located on *3R*, but only 8.5% were in the region spanned by *3RP* (Table S1). The fractions of DEG relative to all genes on *3R* (1.11%) or within *3RP* (1.22%) were not significantly different from the mean of the other chromosomes (0.88-1.15%), but there was a significant difference between the inversion body and the region of *3R* excluding the breakpoints (1.49 and 0.6%, respectively; Fig. 1H, Table S1). The enrichment of differential expression changes in the region covered by *3RP* was therefore relatively weak, and significant differences in expression between HET and STD occurred throughout the whole genome, as observed already for the comparison between INV and STD.

Such a pattern of transcriptome-wide changes might be explained through the differential expression of one or several transcription factors (TFs) within the inverted genomic region, which could in turn influence the expression of genes (including other TFs) in other genomic regions (see Lavington & Kern 2017; Said et al. 2018 for discussion). Indeed, when we performed gene ontology (GO) term analysis, we found an enrichment of multiple GO terms related to transcriptional regulation (e.g., "Transcription *cis*-regulatory region binding"; "DNA-binding transcription factor activity") and to different developmental processes (Fig. S3). The results of these analyses were qualitatively similar for the comparison INV/INV vs. STD/STD and HET vs. STD/STD (Fig. S3), again underscoring the notion that the INV arrangement behaves like a dominant allele.

In wing discs, by contrast, the INV arrangement tends to be recessive

One of the main adult phenotypic features affected by the *3RP* polymorphism is the overall body size of the flies as well as the relative size of their different body parts, including their wings (e.g., Rako et al. 2006; Kapun et al. 2016a; Durmaz et al. 2018; Paris et al. 2025). To investigate whether the INV arrangement also controls gene expression output in other developmental contexts, we analyzed the transcriptomes of wing discs of wandering third instar larvae. Comparing the STD/STD and INV/INV homokaryotypes, we identified 61 DEG ($p_{adj} \leq 0.001$; Fig. 2A, Table S2). The contribution of *3R* and *3RP* towards differential gene expression was even more pronounced in discs than in embryos, with 56% and 34% of all DEG, respectively (Fig. 2B, Table S2). The fraction of DEG relative to all genes in each genomic region was 0.8% for *3R* and 1.71% within *3RP*, while it ranged between 0 and

0.46% for other genomic regions (Fig. 2G, Table S2). During wing development, transcriptional changes are thus more strongly restricted to *3R* and to the region spanned by *3RP* as compared to other genomic regions than during embryogenesis (see above).

Intriguingly, when performing pairwise comparisons of expression levels between HET and each of the two homokaryotypes, we found a pattern that is completely opposite to that in embryos: in wing discs, the STD arrangement seems to behave like a dominant Mendelian "allele", whereas the INV "allele" seems to be recessive (STD/STD ≈ HET > INV/INV).

25 genes were differentially expressed between HET and INV/INV, while only four genes showed differential expression between HET and STD/STD ($p_{adj} \leq 0.001$; Fig. 2C, E, Table S2), thus suggesting dominance of the STD arrangement over the INV arrangement (contrast the pattern in Fig. 2A and 2E with that in 2C). Increasing the p_{adj} cut-off to 0.1 increased the number of DEG between HET and INV/INV to 523, but only to 23 genes between HET and STD/STD; 398 genes were then differentially expressed between STD/STD and INV/INV (Table S2). A large fraction of genes was shared between the comparisons of HET vs. INV/INV and STD/STD vs. INV/INV (15 genes at $p_{adj} \leq 0.001$; 207 genes at $p_{adj} \leq 0.1$; Fig. 2I). Additionally, 4 DEG were shared between HET vs. STD/STD (16 at $p_{adj} \leq 0.1$), 2 of which were shared between all three comparisons (8 at $p_{adj} \leq 0.1$; Fig. 2I). Again, these findings are corroborated by the analysis of Spearman's rank correlations of gene expression (Fig. S1G).

Genes on *3R* and within *3RP* contributed disproportionately to differential expression in the HET vs. INV/INV comparison, with 44 and 28% of all DEG, respectively (Fig. 2F, H). The low number of DEG makes meaningful comparisons difficult (see Table S2 and Fig. S4 for less stringent p_{adj} cut-offs), but 0.57% of all genes in the region spanned by *3RP* were differentially expressed. This fraction was between 0 and 0.26% for other genomic regions (Fig. 2H, Table S2). When we analyzed GO terms of DEG in wing discs, there was no enrichment of genes involved in transcriptional regulation, as observed in embryos, but instead an overrepresentation of terms related to muscle development and cuticle formation (Fig. S5). These results were similar for the STD/STD vs. INV/INV and HET vs. STD/STD comparisons.

Together, our transcriptomic analyses of embryos and wing discs suggest that differential expression depends strongly on the interaction between *3RP* karyotype and developmental context. As mentioned above, the observed pattern strongly resembles a dominant vs. recessive mode of classical Mendelian inheritance, whereby INV is dominant over STD in

one context but recessive in the other context – below we provide a further, in-depth quantitative analysis of this point. Secondly, while in embryos the observed expression changes are likely to be influenced by several TFs, we did not find any GO enrichment for TFs in wing discs. We therefore assumed that the gene expression changes were largely due to differences at *cis*-regulatory modules of the genes that change expression.

The chromatin landscape of wing discs is controlled by the STD karyotype

To explore the potential involvement of *cis*-regulatory modules (CRMs) in gene regulation in wing discs we used ATAC-seq to identify regions of accessible chromatin which might be indicative of active CRMs. After genome-wide calling of peaks, we identified a total of 23,717 accessible chromatin regions in INV/INV, 24,795 in STD/STD, and 25,087 in HET (Fig. 3, Fig. S6, Table S3). 22,886 peaks were shared between all three karyotypes. These peaks were evenly distributed among the chromosome arms in all karyotypes, even after normalizing for chromosome arm length (Fig. 3A, Fig. S6, Table S3). A small proportion of peaks was uniquely called in each karyotype (3.2% in INV/INV, 3.5% in STD/STD, and 4.5% in HET), while the remaining peaks were found in at least one other karyotype. Interestingly, regions that were called as peaks in one sample but not in another still exhibited accessible chromatin, although not significant enough to be recognized as a peak by MACS2 (Fig. S7).

Normalizing for chromosome arm length, we found that *3R* contains the highest density of unique peaks in both INV/INV (6.86 peaks per million base pairs [Mbp]) and STD/STD (8.88 peaks per Mbp). The other chromosome arms contained between 4.97 and 6.33 peaks per Mbp in INV/INV flies and between 3.44 and 6.37 peaks per Mbp in STD/STD flies (Fig. 3B, C, Table S4). *3RP* exhibited an even higher density of unique peaks both in INV/INV (9.62 peaks per Mbp) and STD/STD (10.83 peaks per Mbp). Surprisingly, HET showed the highest density of unique peaks on the *X* chromosome (16.23 peaks per Mbp), followed by chromosome arm *2L* (9.14 peaks per Mbp) (Fig. 3D, Table S4). This suggests that most peaks on *3R* in HET are shared with one of the two homokaryotypes.

The number of peaks shared between HET and INV/INV but not STD/STD was 537, whereas 1,681 peaks were shared between HET and STD/STD but not INV/INV (Table S5). The largest fraction of shared peaks between HET and INV/INV (22.4%) was observed on the *X* chromosome, while the largest fraction shared between HET and STD/STD (30.3 %) was found on *3R* (Fig. 3E, F, Table S5). In fact, 8.6% of all peaks called in HET that were shared with STD/STD are located on *3R*, while this number was only 2% for INV.

These results suggest that the chromatin accessibility profile in HET wing discs is more similar to STD/STD than to INV/INV, particularly for *3R*. This pattern might underpin the similar expression profiles between HET and STD/STD, in agreement with the notion that the STD chromosomal arrangement has a dominant Mendelian effect upon gene expression in wing discs.

The INV allele is subject to a context-dependent dominance reversal

To formally test whether the INV and STD chromosomal arrangements indeed effectively behave like Mendelian alleles affecting gene expression in a context-dependent dominant vs. recessive manner, we estimated the dominance coefficient *h* for each gene that was differentially expressed in at least one pairwise comparison between the three *3RP* karyotypes (see Materials and Methods for details). Considering genome-wide changes at a p_{adj} cut-off of 0.01, this quantification involved 372 genes in embryos and 182 in wing discs (Fig. 4; Table S6).

The distributions of *h*-values of the DEG in the two developmental contexts are shown in Figure 4A. The two distributions are markedly different from each other (two-sided Kolmogorov-Smirnov test: $p = 3.84 \times 10^{-14}$): the distribution of *h* for embryos is left-skewed (skewness $\gamma = -0.97$) with a mode of 1 (mean = 0.68, median = 0.9, variance $\sigma^2 = 0.16$, kurtosis $\kappa = -0.57$) which implies complete dominance of the INV "allele", whereas the distribution for wing discs is right-skewed ($\gamma = 0.26$) with a mode of 0 (mean = 0.44, median = 0.4, $\sigma^2 = 0.15$, $\kappa = -1.1$) which indicates complete recessivity of the INV "allele" (note that we excluded over- and underdominant genes from statistical analysis since their values were defined to be only nominal; see Materials and Methods). The difference between these two distributions is also evident when comparing the *z*-scores for the different modes of inheritance (Fig. 4B, Table S6).

As our design involved two genetic backgrounds (i.e., two independent sets of INV/IN, STD/STD and HET; see Introduction), the distributions shown in Figure 4 represent *average values* across the two backgrounds. We therefore also sought to analyze the two backgrounds individually (Fig. S8; Table S6). While the individual patterns are more variable, we still observe marked, statistically significant shifts in the distributions of the dominance coefficients between embryos and wing discs for both backgrounds, even though these shifts do not, strictly speaking, involve complete reversals (for one background the mode of *h* shifts between 0.6 and 1.1, while for the other background it shifts between -0.1 and 0.5). Thus, with the caveat in mind that we have examined only two independent sets of STD vs. INV

chromosomes, our results suggest that, *on average*, the INV "allele" is subject to a dominance reversal between embryos and wing discs. More generally, our results document significant *shifts* of the *h*-distributions between the two developmental contexts. This is relevant in view of recent theoretical work suggesting that context-dependent changes in dominance coefficients can broadly stabilize biallelic polymorphism even without complete reversals of dominance, i.e., a substantial fraction of the stabilizing dominance schemes involves *non-reversing* changes (Brud 2025).

While a small number of studies has estimated *h* for genes whose expression is affected by polymorphic inversions (Fuller et al. 2016; Said et al. 2018; Berdan et al. 2021), here we have analyzed how a balanced inversion polymorphism affects the quantitative properties of the *h*-distribution in two different developmental contexts. Our analyses suggest that, as hypothesized, the INV and STD arrangements behave like alternative Mendelian alleles with regard to gene expression and that they are subject to a reversal (or shift) of the degree of dominance between the two contexts.

Owing to this Mendelian behavior, and to the fact that the alternative arrangements not only affect multiple complex fitness-related traits (e.g., Kapun et al. 2016b; Durmaz et al. 2018; Paris et al. 2025) but also the expression of numerous genes in- and outside the inverted region in an apparently concerted manner, the *3RP* polymorphism clearly fulfills all the hallmarks of a polygenic supergene: "*A genetic architecture involving multiple linked functional elements that allows switching between discrete, complex phenotypes in a stable local polymorphism*" (see Thompson & Jiggins 2014, p. 3) and, similarly, "*Supergenes are genomic regions containing sets of tightly linked loci that control multi-trait phenotypic polymorphisms under balancing selection"* (see Gutiérrez-Valencia et al. 2021, p.1; also see Parsons & Bodmer 1961; Schwander et al. 2014; Berdan et al. 2022; Schaal et al. 2022).

Our findings add to a short but growing list of examples of context-dependent dominance reversals (see review in Grieshop et al. 2024). For example, at the gene expression level, previous work has identified dominance reversals in *D. melanogaster*, involving reversals between thermal environments (Chen et al. 2015) or between the sexes (Mishra et al. 2024), but, to the best of our knowledge, no expression-level dominance reversals have so far been documented for chromosomal inversions. At the phenotypic level, three previous studies of inversions in fish, butterflies and seaweed flies have identified potentially beneficial, context-dependent dominance reversals between fitness components and/or between the sexes (Le Poul et al. 2014; Pearse et al. 2019; Mérot et al. 2020). Our observations herein are also interesting in the context of our recent work on the phenotypic effects of the *3RP* inversion

polymorphism where we find evidence for dominance reversals for a size-related trait between the sexes as well as between different traits (Paris et al. 2025). In addition, another trait, desiccation resistance, exhibits a sex-dependent non-reversing change of dominance (INV being recessive in females, but HET overdominant in males) (Paris et al. 2025).

When assessing which genes show actual evidence of a dominance reversal, we found that only a handful exhibit significantly differential expression in *both* embryos *and* wing discs and hence allowed for a comparison of dominance coefficients (Fig. S9). Interestingly, this suggests that the INV "allele" is subject to a "higher-order" dominance reversal (or shift) that involves largely distinct sets of genes with inherently different *h*-distributions between the two developmental contexts. The only gene that showed a clear reversal of dominance between embryos and wing discs is *Lysine demethylase 2* (*Kdm2*). This gene encodes a histone demethylase that plays a major role in gene regulation and the histone code (Lagarou et al. 2008; Kavi & Birchler 2009; https://flybase.org/reports/FBGN0037659); it could therefore be an important factor in mediating the global context-specific gene expression changes which we have observed in our data.

What causes dominance reversals? Theory suggests that dominance reversals might reflect nonlinear properties of genotype-phenotype (G-P), phenotype-fitness or genotype-fitness maps, i.e., they might arise when context-dependent (fitness or G-P) functions overlap in the vicinity of their concave (fitness or trait) optima (e.g., see Connallon & Chenoweth 2019; Reid et al. 2022; Grieshop et al. 2024). The nonlinearity of such maps is conceptually closely related to Wright's physiological model of dominance, which posits a saturating (concave) relationship between gene (enzyme) activity and phenotype (fitness) (Wright 1929, 1934, 1977), and modern extensions and reformulations of this model (e.g., Kacser & Burns 1981; Keightley 1996; Gilchrist & Nijhout 2001; Bagheri & Wagner 2004; Bagheri 2006; Veitia et al. 2013). Such nonlinearity also plays a role in evolutionary models of dominance postulating that selection optimizes gene expression so that higher expression levels (or enzyme concentrations) might buffer against the effects of environmental perturbations and deleterious loss-of-function mutations (Hurst & Randerson 2000; Huber et al. 2018; also see Manna et al. 2011), an idea dating back to Haldane (1930).

Another possibility is that dominance reversals result from the action of another locus, a "dominance modifier", which affects the dominance properties of the focal locus (Spencer & Priest 2016; Otto & Bourguet 1999). The idea that dominance modifiers underlie the evolution of dominance was developed by Fisher (1928) but criticized by Wright (1929), who argued that selection on modifier loci would likely be too weak to be effective – this prompted

Wright to develop his physiological model (see above). Indeed, several empirical observations (e.g., the commonly observed negative relationship between *h* and the selection coefficient *s*) seem to be at odds with Fisher's theory for the evolution of dominance, so that it is widely held to be incorrect, or at least in a large part (e.g., see Orr 1991; Charlesworth & Charlesworth 2010; Billiard & Castric 2011; Huber et al. 2018; Di & Lohmueller 2024). However, the potential importance of dominance modifiers should not be dismissed too readily.

First, there is long-standing evidence for genetic variation affecting dominance that is consistent with the existence of dominance modifiers (e.g., Fisher 1938; reviewed in Sved & Mayo 1970; Mayo & Bürger 1997; Otto and Bourguet 1999). Similarly, mounting evidence indicates that the dominance properties of focal loci often vary as a function of genetic background and multi-locus interactions (reviewed in Li & Bank 2023).

Second, there is a large body of indirect (and, more rarely, direct) evidence for the existence of dominance modifiers and their evolution, for example from selection and/or QTL experiments (e.g., Ohh & Sheldon 1970; Thomson & Thoday 1972; Day & Cummins 1975; McKenzie & Game 1987; Doebley et al. 1995; Davies et al. 1996; Mayo & Bürger 1997; Bourguet et al. 1997; Bourguet 1999; Otto & Bourguet 1999; Naisbit et al. 2003). The perhaps best-known direct evidence comes from the discovery of a *trans*-acting small non-coding RNA (sRNA) that determines the dominance relations of alleles at the *SP11* locus involved in self-incompatibility in *Brassica* (Tarutani et al. 2010) – this represents *prima facie* molecular evidence for the sort of dominance modifier envisaged by Fisher (1928; see Billiard & Castric 2011). Intriguingly, in a similar vein, Durand et al. (2014) identified a complex regulatory network consisting of 17 sRNA-producing loci and their targets which together control a dominance hierarchy of alleles at the self-incompatibility locus in *Arabidopsis halleri*. For a recent example of molecularly identified dominance modifiers in yeast see the study by Matsui et al. (2022).

Third, in situations where heterozygotes are maintained at appreciable frequencies, for example by migration-selection balance or short-term balancing (e.g., overdominant or antagonistic balancing) selection, theory shows that selection can favor dominance modifiers (Otto & Bourguet 1999; Spencer & Priest 2016; also cf. Van Dooren 1999; Billiard & Castric 2011; Proulx & Teotónio 2022) which may or may not eventually lead to long-term balancing selection. This point is critical as Wright's objection to Fisher's theory was based on the rarity of heterozygotes and, as pointed out by Wright himself (Wright 1929), does not apply to

balanced polymorphisms (see Otto & Bourguet 1999; Bagheri & Wagner 2004 for discussion).

Notably, Fisher's and Wright's opposing views may not necessarily be mutually exclusive (Gilchrist & Nijhout 2001; Bagheri 2006; Plutynski 2008; Billiard et al. 2021). Mechanistic models investigating the regulation of gene expression indicate that the two viewpoints might be reconcilable (Omholt et al. 2000; Llaurens et al. 2015; Fyon et al. 2015; Porter et al. 2017; Grieshop et al. 2024; reviewed in Billiard et al. 2021). Porter and colleagues (2017), for example, developed a biophysically explicit model of gene regulation and found that in heterozygotes molecular interactions between TF variants that are competing for binding to their shared promoters can lead to the emergence of dominance (also see Porter et al. 2025). Interestingly, Grieshop et al. (2024) have recently extended the model of Porter et al. (2017), providing the first biophysically explicit model of a dominance modifier and its evolution. In short, this model involves a dominance-reversed TF and requires molecular interactions between its *cis*-regulatory binding sites and a *trans*-acting regulatory stimulus. Simulations show that the focal (sexually antagonistic) polymorphism (i) can be maintained by the dominance reversal and (ii) exhibits reversed allele-specific expression between the two contexts (i.e., sexes) (Grieshop et al. 2024).

As several of the empirical and theoretical advances discussed above indicate that transcriptional regulators such as TFs or sRNAs might represent promising candidates for dominance modifiers, we sought to take a closer look at the transcriptional regulators in our dataset and to see whether we could discern patterns that might be consistent with the model of Grieshop et al. (2024).

The transcriptional regulator *HP1c* might be a dominance modifier

The fact that we found enrichment of several GO terms related to transcriptional regulation in embryos (where INV tends to be dominant), but not in wing discs (where INV tends to be recessive; Fig. S3, Fig. S5) is interesting since *trans*-acting variants are more often dominant or recessive as compared to *cis*-regulatory variants which have predominantly additive effects (Hughes et al. 2006; Wray 2007; Lemos et al. 2008; McManus et al. 2010; Zhang et al. 2011; Gruber et al. 2012; Meiklejohn et al. 2014; Porter et al. 2017).

In embryos, a quarter (n=31) of all DEG ($p_{adj} \leq 0.001$) between INV/INV and STD/STD encode transcriptional regulators (Table S7). Notably, most of these genes were expressed at a lower level in STD, except for *HP1c*. Three of the 31 loci, *abd-A*, *Eip93F* and *HP1c*, are located in the region spanned by *3RP*. While none of the 4 DEG between HET and INV/INV

encodes a transcriptional regulator, 22% (*n*=37) of the DEG between HET and STD/STD do. Twenty-five of these genes are the same as in the INV/INV vs. STD/STD comparison, and expression was likewise lower in STD/STD for all genes except *pre-mod(mdg4)-P*. These similarities between the INV/INV vs. STD/STD and the HET vs. STD/STD comparisons corroborate that expression in HET is largely determined by the dominant INV chromosomal arrangement. Also here, in the HET vs. STD/STD comparison, three genes are located in the region spanned by *3RP*, namely *abd-A*, *Eip93F* and *pre-mod(mdg4)-P*. *3R* and *3RP* contributed 38.4% and 14.4%, respectively, of the total number of DEG between the INV/INV and STD/STD in embryos, while these proportions were substantially higher in wing discs (56% of all DEG on *3R* and 34% in the region of *3RP*; Tables S1 and S2). We only found a single differentially expressed transcriptional regulator in wing discs (both in the STD/STD vs. INV/INV and the HET vs. INV/INV comparisons), namely *HP1c*. When comparing differential expression between embryos and discs, we only found four DEG that were shared between the two developmental contexts ($p_{adj} \leq 0.001$): *CG18853, CG31251*, *asRNA:CR46029,* and, interestingly, again, *HP1c*.

This latter gene, *Heterochromatin Protein 1c*, encodes a chromatin-binding protein involved in telomere capping and transcription regulation and forms a transcriptional complex with two zinc-finger TFs, *woc* and *row* (http://flybase.org/reports/FBgn0039019.htm; Font-Burgada et al. 2008; Abel et al. 2009; Kwon & Workman 2011; Kessler et al. 2015; Di Mauro et al. 2020; Sun et al. 2021; Schoelz et al. 2021; Schoelz & Riddle 2022). *HP1c* acts mostly as a transcriptional activator (Kwon et al. 2010; Kwon & Workman 2011; Schoelz et al. 2021; Schoelz & Riddle 2022) and genes bound by HP1c are typically expressed at a higher level than unbound genes across chromatin contexts (Schoelz et al. 2021). This is interesting given that selection might favor higher (optimal) expression levels, and stronger dominance, unless the costs of higher expression are too large (see Hurst & Randerson 2000; also see Haldane 1930; Manna et al. 2011; Huber et al. 2018).

Five facts render *HP1c* an attractive candidate for a dominance modifier: *HP1c* (i) is the only differentially expressed transcriptional regulator shared between embryos and wing discs; (ii) functions as a epigenetic regulator of transcription, causing higher expression of genes bound by it; (iii) can, depending on the context, act as a repressor (Sun et al. 2021); (iv) exhibits foci often corresponding to sites where many TFs or other regulatory proteins are co-localized, i.e., so-called “hotspots” which are typically located in or near highly active genes (Moorman et al. 2006; de Wit et al. 2007); and (v) harbors single nucleotide polymorphisms (SNPs) that represent outliers in terms of genetic differentiation (F_{ST}) between *3RP* INV vs. STD chromosomes, as shown in our previous work (Kapun et al. 2023).

The latter observation is confirmed by an analysis of phased DNA sequencing data for the INV vs. STD lines used here (Fig. S10): indeed, *HP1c* harbors several SNPs that are strongly differentiated between the INV and STD arrangement, with a maximum value of F_{ST} = 0.58 (cf. Kapun et al. 2023). Closer inspection of the gene-dense region surrounding *HP1c* also revealed other loci with F_{ST} outliers; in particular, strongly differentiated SNPs at the *Usp12-46* locus (*3R* 22,752,448; maximum F_{ST} = 0.92), located about 7 kb upstream of *HP1c* (Fig. S10). Again, we had singled out this locus as an outlier of F_{ST} differentiation between the INV and STD arrangements in previous work (Kapun et al. 2016a, 2023). Similar to the role of *HP1c* as a Notch repressor (Sun et al. 2021), *Usp12-46* encodes a deubiquitinating enzyme that negatively regulates Notch (Moretti et al. 2012). Furthermore, *Usp12-46* positively regulates Wnt signaling (Spencer et al. 2023).

While the promoters of several genes in the region shown in Fig. S10, including those of *HP1c* and *Usp12-46*, are clearly accessible, chromatin accessibility does not differ markedly between the three *3RP* karyotypes, with one major exception: a small region upstream of the *Usp12-46* promoter (see the black rectangle in Fig. S9), where chromatin is less accessible in INV/INV as compared to STD/STD and HET (i.e., consistent with STD being dominant in wing discs) and which also harbors SNPs that are major F_{ST} outliers. This region could thus represent a CRM, potentially of both *HP1c* and *Usp12-46*. It is noteworthy in this context that in the biophysical model of Grieshop et al. (2024) the dominance modifier, represented by a TF polymorphism, exhibits molecular interactions between its *cis*-regulatory binding sites and a *trans*-acting regulatory stimulus.

The *HP1c* locus and its neighborhood thus represent a promising genomic region that might underlie the dominance reversal between the *3RP* INV and STD arrangements and their context-dependent effects on expression. (Another interesting candidate gene is *Kdm2*, as mentioned further above.)

(For analyses of (i) indels in putative CRMs that might affect chromatin accessibility and (ii) overrepresented sequence motifs that might represent preferred TF binding sites and promoter elements, we refer to the supplementary results, Tables S8, S9, and S10, and Fig. S10 in the Supplementary Materials file.)

Quantification of *cis* versus *trans* effects

Given the likely involvement of transcriptional regulators in the observed gene expression differences between the STD and INV chromosomal arrangements, we sought to quantify the

relative contribution of *cis* vs. *trans*-regulatory genetic changes to expression differences between the INV and STD karyotypes. In the context of inversion polymorphism, allelic imbalance (i.e., an expression difference between the INV and STD alleles in the heterokaryotype) implies that a genetic difference between the parental karyotypes (INV/INV and STD/STD) affecting gene expression is in linkage disequilibrium (LD) with the gene whose expression is affected (see Wang et al. 2020; also cf. Rockman & Kruglyak 2006; Wittkopp et al. 2008; Signor & Nuzhdin 2018). As LD typically decays quite rapidly with physical distance, this is interpreted as a "*cis*" ("nearby" or "local") effect, i.e., differential gene expression is due to a linked *cis*-regulatory polymorphism. In contrast, no (or weak) allelic imbalance in the heterokaryotype implies a "*trans*" ("distant") genetic effect. In such a situation, an expression difference between the INV/INV and STD/STD parental karyotypes is interpreted as being independent of any differences in *cis*-regulatory elements between the karyotypes and instead caused by a genetically based effect which is not in LD with the gene whose expression is affected (see Wang et al. 2020). This can arise, for instance, from genetic differences in the binding specificity or expression levels of a transcription factor. There can also exist a mix of such *cis* and *trans* effects (Wang et al. 2020).

Figure 5 shows the quantification of *cis* vs. *trans* effects in embryos and wing discs for (i) all genes and (ii) those genes that are spanned by *3RP.* We calculated *cis*-regulatory differences as the $\log_2$ of the ratio of allelic expression in the STD / INV heterokaryotypes ($\log_2$ fold-change [STD / INV] in HET), and the expression difference between the parental homokaryotypes was calculated as the $\log_2$ of the ratio of total transcript abundance between them ($\log_2$ fold change [STD homokaryotype / INV homokaryotype]) (e.g., see Wittkopp et al. 2008). We used HyLiTE to assign reads in the heterokaryotypes to the parent-of-origin STD vs. INV alleles (see Duchemin et al. 2015; Wang et al. 2020) and we followed the system by Shi *et al.* (2012) to classify them into the categories "only *cis*", "only *trans*", "*cis-trans*" (*i.e.*, a combination of *cis* and *trans* effects), and "no *cis-trans*". Briefly, DEG in the STD/STD vs. INV/INV comparison that showed a similar differential allelic expression were classified as only *cis*. DEG for which the two alleles in HET showed no differential expression were classified as only *trans*. Otherwise (i.e., if a gene and its alleles in the F1 showed differential expression but the log2 fold changes were dissimilar), genes were classified as *cis-trans*. Those genes for which *cis*-regulatory differences account for all of the expression differences between the INV and STD alleles (i.e., 100% *cis*) would fall on the diagonal 1:1 ($y = x$) line, whereas genes whose expression is completely unaffected by any *cis*-regulatory effects between the INV and STD alleles (0% *cis* = 100% *trans*) would fall on the horizontal ($y = 0$) line (see Wittkopp et al. 2008).

As can be seen from Figure 5 and the quantification in Table S11, most genes for which we can assign reads allele-specifically (4923 in embryos; 4144 in wing discs) do not show clear *cis* only or *trans* only effects ("no *cis*-*trans*": 90.92% in embryos, 88.15% in wing discs). For the remaining genes, *trans* only effects tend to outweigh *cis* only effects in both embryos (Fig. 5A) and wing discs (Fig. 5B): 309 genes in embryos (6.28%) and 308 genes in wing discs (7.43%) were classified as *trans* only, whereas 64 genes in embryos (1.3%) and 75 genes in wing discs (1.81%) were classified as *cis* only. This stronger *trans* effect is also apparent by the fact that the Pearson's correlation coefficient *R* of gene and allele expression changes is close to zero ($R = 0.05$ in embryos; $R = 0.08$ in wing discs). Genes with a significant difference in both gene and allele expression ($p \leq 0.05$) showed a much higher degree of correlation, with $R = 0.29$ in embryos and even $R = 0.82$ in wing discs. Mixed *cis* and *trans* effects were found for 76 genes in embryos (1.54%) and 110 genes in wing discs (2.65%).

When specifically considering genes spanned by *3RP* (Fig. 5C, Fig. 5D), there is a higher degree of correlation in both embryos ($R = 0.12$) and wing discs ($R = 0.1$) as compared to Fig. 5A and Fig. 5B, indicating more *cis* effects than when considering all genes. Indeed, while the share of *cis* only genes in *3RP* is higher than when assessing all genes in both embryos and wing discs, the share of *trans* genes only increases in wing discs: 18 out of 609 genes in embryos (2.96%) and 29 out of 522 in wing discs (5.56%) showed *cis* effects while 30 genes in embryos (4.93%) and 51 genes in wing discs (9.77%) exhibited *trans* effects. This could indicate that a higher proportion of genes in *3RP* change expression due to genetic differences at *cis*-linked regulatory elements, while genes in the rest of the genome are more likely to change expression due to genetic differences in *trans*, *i.e.*, likely differences in the expression of upstream TFs.

As the above results represent *averages* across two backgrounds (see above), and as background-specific effects in *cis*/*trans* regulation have been documented in *Drosophila* before (Osada et al. 2017; Puixeu et al. 2023; Glaser-Schmitt et al. 2024), we were interested in quantifying these effects individually for the two backgrounds (Fig. S11, Table S11). This analysis confirmed that, for each of the two backgrounds, the majority of genes does not show any clear *cis* only or *trans* only effects. Secondly, for genes that do show *cis* only vs. *trans* only effects, the backgrounds differ markedly in the number and proportion of *cis* vs. *trans* effects (Table S11). Thus, the observation that *trans* effects outweigh *cis* effects in both embryos and wing discs holds true *on average*, but there are clearly marked effects of genetic background.

Summary and Conclusions

Population genetics theory suggests that beneficial dominance reversals, the phenomenon whereby an allele is partially or completely dominant in contexts in which it is selectively favored but recessive in contexts in which it is deleterious, might be an important mechanism of balancing selection, yet empirical evidence remains relatively scarce (see Connallon & Chenoweth 2019; Grieshop et al. 2024). Moreover, recent theoretical work suggests that even non-reversing shifts in the degree of dominance between different contexts can contribute to stabilizing biallelic polymorphism (Brud 2025).

Here, we have used RNA-seq and ATAC-seq to examine how a globally widespread chromosomal inversion polymorphism in *D. melanogaster*, *In(3R)Payne*, thought to be maintained by long-term balancing selection (e.g., Kapun et al. 2023; also see Paris et al. 2025), affects gene regulatory profiles during development. We find that the alternative chromosomal arrangements of this inversion polymorphism behave like classical Mendelian alleles and that (*on average*, across two independent genetic backgrounds assayed) they undergo a major context-dependent reversal of dominance: while in embryos the INV arrangement tends to have dominant effects on the expression of most genes, in wing discs the STD arrangement tends to behave dominantly. More generally, when looking at the individual genetic backgrounds, we observe significant shifts between embryos and wing discs in the distribution of the dominance coefficients with respect to the INV “allele” (cf. Brud 2025).

While the relationship between patterns of gene expression and chromatin accessibility documented here and Darwinian fitness remains unclear, our results are consistent with a role of dominance reversals (or shifts in dominance) in maintaining balanced inversion polymorphisms (see Paris et al. 2025; also see Mérot et al. 2020).

It is conceivable that the observed changes in the dominance distributions between the two developmental contexts might be mediated by a dominance modifier (Fisher 1928, 1930a,b), for example a TF or another transcriptional regulator (Grieshop et al. 2024). Consistent with Fisher’s concept and with a small handful of studies that have established the existence of molecularly defined dominance modifiers (Tarutani et al. 2010; Durand et al. 2014; Matsui et al. 2022), we identify a major epigenetic transcriptional regulator, the chromatin binding protein *HP1c*, as a promising candidate dominance modifier.

It is worth pointing out in this context that one of the main objections to Fisher’s modifier theory was based on the presumed rarity of heterozygotes (Wright 1929, 1934). If, however,

heterozygotes are relatively common, say, e.g., due to some form of short-term balancing selection, selection can favor dominance modifiers, as has been found in several theoretical studies (Otto & Bourguet 1999; Spencer & Priest 2016; also see Proulx & Teotónio 2022), and this might or might not eventually lead to a long-term balanced polymorphism. A long-term balanced chromosomal inversion polymorphism which shows evidence of dominance reversals at both the gene expression level (this study) as well as at the fitness-component level (see Paris et al. 2025) might be compatible with such a scenario.

Further studies of balanced inversion polymorphisms in the light of dominance reversals (or context-dependent shifts in dominance coefficients more generally) will undoubtedly be of great interest and might help to illuminate how they are being maintained at intermediate frequencies in natural populations.

Materials and Methods

Drosophila stocks and maintenance

We used four isofemale lines derived from two populations in southern Florida (USA): Jacksonville and Homestead, previously collected by one of us (PS) (Table S11; also see Kapun et al. 2016b). These lines were chosen because they carried both the INV and STD arrangement of *3RP*, but not any other segregating cosmopolitan inversion polymorphisms [(*In(2L)t*, *In(2R)Ns*, *In(3R)K*, *In(3R)Mo*; see Lemeunier and Aulard (1992); Kapun and Flatt (2019)]. The presence of inversions was determined using karyotype-specific PCR markers (Matzkin et al. 2005; Corbett-Detig et al. 2012) on DNA extracted from pools of 10-15 flies. Stocks were maintained under controlled conditions at 18°C with ~60% relative air humidity and a 12-hour light:dark cycle on a standard cornmeal-sugar-yeast-agar diet.

Isolation of inverted and standard chromosomes

To isolate individual INV or STD *3R* chromosomes, we followed the chromosome extraction protocol of Kapun et al. (2016b). In short, for each isofemale line we performed five replicate crosses between single males and 3-5 females of the third-chromosome *TM6B* balancer stock (Bloomington Drosophila Stock Center [BDSC] #1711). Balanced stocks were backcrossed four times to the balancer stock to ensure genetic homogeneity.

Generation of inversion homo- and heterokaryotypes

We created *3RP* INV/INV and STD/STD homokaryotypes by crossing previously isolated *3R* chromosomes (maintained over the balancer) in a pairwise manner, resulting in all 16 possible combinations of isolated chromosomes within each karyotype (including reciprocal

crosses; e.g., for the 4 INV lines 1-4: 12, 13, 14, 23, 24, 34, 21; 31, 41, 32, 42, 43; and 11, 22, 33, 44; and analogously for STD lines 5-8; see Table S11). To avoid inbreeding and maximize allelic variation within the inverted region, we selected two heterozygous INV homokaryotypes (23 and 41) and their corresponding STD counterparts (67 and 85; Table S11). We maintained these four heterozygous homokaryotypes as separate panmictic populations (in small population cages at 25°C, ~60 % RH, 12-h LD, on a standard cornmeal-sugar-yeast-agar diet) and let them outbreed and recombine for 22 generations. From these homokaryotypes we generated two heterokaryotypes (HET = INV/STD) by reciprocally crossing the recombinant homokaryotypes (F22 adults) 23 (INV) to 67 (STD), and 41 (INV) to 85 (STD). This allowed us to partially recreate the original chromosomal combinations found in the isofemale lines (Table S10; chromosomes 1 and 5; 2 and 6; 3 and 7; and 4 and 8 originate from the same line, respectively). We used embryos and larvae of the resulting F1 HET individuals for subsequent analysis, together with the F23 progeny of the parental recombinant homokaryotypes.

RNA-seq

By treating the alternative INV vs. STD arrangements as Mendelian alleles, we used RNA-seq expression profiling to study allele-specific (i.e., arrangement-specific) patterns of gene expression. For each of the four homokaryotypes and the two heterokaryotypes we extracted RNA in duplicate reactions. Twelve wing discs from twelve L3 larvae per replicate were dissected in PBS on ice and flash frozen in liquid nitrogen. For HET, reciprocal cross directions were pooled (e.g., six samples from 41 x 85 pooled with six samples from 85 x 41). For embryo samples, we collected 20-24 eggs 4-6 h after egg laying per replicate, and also here reciprocal cross directions were pooled. Total RNA was extracted using a Promega Maxwell RSC simplyRNA Tissue Kit and Maxwell RSC Instrument, following the manufacturer's protocol. TruSeq stranded mRNA-seq library preparation and sequencing on an Illumina NovaSeq (50 bp paired-end) were carried out by Edinburgh Genomics (https://genomics.ed.ac.uk/). Each library was sequenced twice to a depth of between 14 and 38 Million (mean: 21.5 M) reads. Reads were analyzed with reference to the *D. melanogaster* reference genome dm6 using Salmon (Patro et al. 2017) and differential expression analysis was performed using DESeq2 (Love et al. 2014). Fold changes in gene expression were adjusted using the “apeglm” method (Zhu et al. 2019). When comparing different regions on chromosome *3R*, the extended breakpoint regions of *3RP* were defined as the left (3R:16,432,209) and right (3R:24,744,010) breakpoint ± 2 Mb (also see Matzkin et al. 2005; http://flybase.org/reports/FBab0005639.htm). Gene ontology (GO) analysis was performed using ShinyGO 0.76 (Ge et al. 2020) with default settings. For data visualisation

and plotting in R (R Core Team, 2023) and RStudio we used the following packages: EnhancedVolcano (Blighe et al. 2018), ggplot2 (Wickham 2016), tidyverse (Wickham et al. 2019), dplyr (Wickham et al. 2023), tibble (Müller & Wickham 2023), tximport (Soneson et al. 2015), magrittr (Bache & Wickham 2022), and RColorBrewer (Neuwirth 2022).

Estimation of the dominance coefficient *h*

We removed *X*-linked genes for this analysis as we did not determine the sex of either embryos or larvae before sample processing. For genes that were significantly differentially expressed in at least one pairwise comparison between the three karyotypes, we estimated the degree of dominance or dominance coefficient *h* (e.g., see Lagervall 1961; Falconer & Mackay 1996; Lynch & Walsh 1998; Di & Lohmueller 2024) by taking the mean of the normalized read counts (MNRC) after DeSeq2 analysis for each karyotype and using the following formula for *h* (following Bourguet et al. 2000; cf. Roux et al. 2005; Paris et al. 2008):

h = (MNRC [STD/STD] - MNRC [HET]) / (MNRC [STD/STD] - MNRC [INV/INV])

(see Fuller et al. 2016 for a similar analysis in the context of inversions).

We rounded the values to the first decimal. Using this equation, the inverted (INV) karyotype ("allele") is defined as being dominant when $h = 1$ (i.e., meaning STD is recessive), as additive (semi-dominant) when $h = 0.5$, and as recessive when $h = 0$ (i.e., meaning that STD is dominant). In our empirical expression data, we allowed for deviations of *h* of ± 0.1 and hence operationally classified the INV allele as being fully dominant when $0.9 \leq h \leq 1.1$, as partially dominant when $0.6 < h < 0.9$, as fully additive when $0.4 \leq h \leq 0.6$, as partially recessive when $0.1 < h < 0.4$, and as fully recessive when $-0.1 \leq h \leq 0.1$. We also quantified the number of differentially expressed genes (DEG) that showed overdominance ($h > 1$) or underdominance ($h < 0$) by taking $h > 1.1$ to indicate overdominance and $h < -0.1$ to indicate underdominance. Note that, here, the classification of DEG into the categories dominant vs. recessive, additive, overdominant or underdominant does not refer to selection or fitness, as is usually the case in population genetics (e.g., see Di & Lohmueller 2024), but to the (phenotypic) mode of inheritance, as is the convention in classical transmission genetics (Zschocke et al. 2022; also see Gibson et al. 2004 and Fuller et al. 2016). For the generation of plots see the section on RNA-seq above; additionally, we used the R package ggdist (Kay 2023). To visualize over- and underrepresentation of the dominance categories in each dataset, we calculated z-scores for the numbers of genes that fall into each category (for a similar analysis see Fuller et al. 2016).

ATAC-seq

We collected twelve wing discs per replicate and processed two technical replicates for each karyotype to analyze chromatin accessibility using ATAC-seq ("assay for transposase-accessible chromatin with sequencing"; see Buenrostro et al. 2013, 2015). Discs were dissected in PBS and kept on ice until tagmentation. The Omni-ATAC protocol (Corces et al. 2017) was followed for tagmentation and library preparation. The library preparation PCR was run for 15 cycles, libraries were analyzed on Agilent 2200 TapeStation and sequenced on an Illumina NovaSeq (50 bp paired-end) by Edinburgh Genomics. Each library was sequenced twice to a depth of between 42 and 68.5 Million (mean: 54 M) reads. Reads were aligned to the *D. melanogaster* genome 6.36 (FlyBase; https://flybase.org/) using bowtie2 v.2.3.4.1 (Langmead and Salzberg 2012) with the options --very-sensitive -X 2000 and converted into bam files using samtools v.1.7 (Danecek et al. 2021). Alignment rates ranged between 79 and 95 % (mean: 89.3 %). We also used samtools to remove non-unique alignments and alignments with mapping quality of less than 30, to filter out reads mapping to the mitochondrial genome, to remove PCR duplicates, and to merge technical replicates. bedtools v.2.29.2 (Quinlan and Hall 2010) was used to convert the bam into bed files and reads were shifted according to Buenrostro et al. (2013). Peaks were called using macs2 (Zhang et al. 2008) with the following options: --nomodel --shift -100 --extsize 200 -g dm --keep-dup all. Data visualization was performed using deeptools v.3.5.0 (Ramírez et al. 2016) and pyGenomeTracks v.3.6 (Ramírez et al. 2018; Lopez-Delisle et al. 2021). Motif analysis was performed using XSTREME (Grant & Bailey 2021) from the MEME Suite 5.4.1 (Bailey et al. 2015) with default parameters and the *Any number of repetitions* option in the MEME analysis. For generation of other plots see "RNAseq" and "Estimation of the dominance coefficient *h*". Finally, as a methodological caveat, we note that TF binding sites of silencer-associated TFs might not be detectably open/accessible by ATAC-seq assays (Hofbauer et al. 2024).

Allele-specific expression analysis and estimation of *cis* and *trans* effects

We first generated a gene set containing all genes from the *D. melanogaster* reference genome dm6 and aligned RNAseq reads to it using bowtie2 v 2.4.1 (Langmead and Salzberg 2012) with the option --very-sensitive-local. We then used samtools v 1.21 (Danecek et al. 2021) to generate a pileup file according to the HyLiTE manual (Duchemin et al. 2015) and finally used HyLiTE version 2.0.2 to assign RNA-seq reads from HET samples allele-specifically to the INV or STD genotype. This was done for each HET genotype (23X67 and 41X85) individually. During the analysis, we removed genes without allele-specific

information as well as X-linked genes since we did not sex larvae before dissection. We normalized the allele-specific read counts by the total read counts for each gene and calculated the mean of the two genotypes for the STD vs. INV comparison. To enable direct comparison of allele-specific expression changes in HET with expression changes of genes in the STD vs. INV comparison, we used normalized read counts that were generated during the DESeq2 analysis and calculated $\log_2$ fold changes and *p*-values for alleles and for genes. *p*-values were also calculated to test for a significant difference between the two $\log_2$ fold change values for each gene. To categorize genes into "no *cis-trans*", "*cis* only", "*trans* only", and "*cis-trans*", we followed the pipeline by Shi *et al.* (2012): We first tested if the two $\log_2$ fold change values for a gene were significantly different from one another ($p \leq 0.05$). If they were different and the alleles showed no differential expression ($p > 0.05$), a gene was classified as "no *cis-trans*". If they were different and there was also a difference in allelic expression ($p \leq 0.05$), a gene was classified as "*cis* only". If the two $\log_2$ fold change values for a gene were similar ($p > 0.05$) and the alleles were similar in their expression ($p > 0.05$), a gene was classified as "*trans* only". Finally, if the change in gene expression and allelic expression was similar and the alleles were differentially expressed ($p \leq 0.05$) a gene was classified as "*cis-trans*". Plots were generated with ggplot2 v 3.5.1 (Wickham 2015).

Data availability

The ATAC and RNA-seq data are available from the European Nucleotide Archive (ENA) under accession PRJEB83419 and PRJEB83014, respectively.

Author contributions

Definitions according to CRediT (https://casrai.org/credit/): EDM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing; EK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing; PS: Resources, Writing – review & editing; TF: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing; SK: Conceptualization, Funding acquisition, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

Our work was supported by grants from the Natural Environment Research Council (NERC;

grant NBAF1214 to SK), the European Molecular Biology Organization (EMBO; long-term fellowship ALT 248-2018 to EK), and the Swiss National Science Foundation (SNSF; grants 31003A_182262; 310030_219283; and FZEB-0-214654 to TF).

Acknowledgements

We thank three anonymous reviewers, the associate editor, and Alistair P. McGregor for valuable feedback on our manuscript. We are grateful to the team of Edinburgh Genomics at the University of Edinburgh for sequencing our samples; this center is partly supported by core funding from NERC (UKSBS PR18037). We also thank the Bloomington Drosophila Stock Center (BDSC, Bloomington, Indiana, USA) for providing fly stocks (the BDSC is supported by NIH grant P40OD018537).

References

Abel, J., R. Eskeland, G. D. Raffa, E. Kremmer and A. Imhof, 2009 *Drosophila* HP1c is regulated by an auto-regulatory feedback loop through its binding partner Woc. *PLoS One* 4:e5089.

Andrés, A. M., M. J. Hubisz, A. Indap, D. G. Torgerson, J. D. Degenhardt et al., 2009 Targets of Balancing Selection in the Human Genome. *Molecular Biology and Evolution* 26: 2755-2764.

Arnqvist, G., and L. Rowe, 2023 Ecology, the pace-of-life, epistatic selection and the maintenance of genetic variation in life-history genes. *Molecular Ecology* 32: 4713-4724.

Ayala, F. J., and C. A. Campbell, 1974 Frequency-Dependent Selection. A*nnual Review of Ecology and Systematics* 5: 115-138.

Bache, S., and H. Wickham, 2022 magrittr: A Forward-Pipe Operator for R. (https://magrittr.tidyverse.org, https://github.com/tidyverse/magrittr).

Bagheri, H. C., 2006 Unresolved boundaries of evolutionary theory and the question of how inheritance systems evolve: 75 years of debate on the evolution of dominance. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 306B:329-359.

Bagheri, H. C., and G. P. Wagner, 2004 Evolution of dominance in metabolic pathways. *Genetics* 168:1713-1735.

Bailey, T. L., J. Johnson, C. E. Grant and W. S. Noble, 2015 The MEME Suite. *Nucleic Acids Research* 43:W39-49.

Barson, N. J., T. Aykanat, K. Hindar, M. Baranski, G. H. Bolstad et al., 2015 Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. *Nature* 528: 405-408.

Barton, N., and P. Keightley, 2002 Understanding quantitative genetic variation. *Nature Reviews Genetics* 3: 11-21.

Barton, N. H., and M. Turelli, 1989 Evolutionary quantitative genetics: how little do we know? *Annual Review of Genetics* 23: 337-370.

Berdan, E. L., T. Flatt, G. M. Kozak, K. E. Lotterhos, and B. Wielstra, 2022 Genomic architecture of supergenes: connecting form and function. *Philosophical Transactions of the Royal Society B* 377 20210192.

Berdan, E. L., C. Mérot, H. Pavia, K. Johannesson, M. Wellenreuther, et al., 2021 A large chromosomal inversion shapes gene expression in seaweed flies (*Coelopa frigida*). *Evolution Letters* 5: 607-624.

Berdan, E. L., N. H. Barton, R. Butlin, B. Charlesworth, R. Faria et al., 2023 How chromosomal inversions reorient the evolutionary process. *Journal of Evolutionary Biology* 36: 1761-1782.

Bergland, A. O., E. L. Behrman, K. R. O'Brien, P. S. Schmidt and D. A. Petrov, 2014 Genomic Evidence of Rapid and Stable Adaptive Oscillations over Seasonal Time Scales in *Drosophila*. *PLoS Genetics* 10: e1004775.

Bertram, J., and J. Masel, 2019. Different mechanisms drive the maintenance of polymorphism at loci subject to strong versus weak fluctuating selection. *Evolution* 73:883-896.

Billiard, S., and V. Castric, 2011 Evidence for Fisher's dominance theory: how many 'special cases'? *Trends in Genetics* 27:441-445.

Billiard, S., V. Castric and V. Llaurens, 2021 The integrative biology of genetic dominance. *Biological Reviews* 96:2925-2942.

Bitarello, B. D., D. Y. C. Brandt, D. Meyer, and A. M. Andrés, 2023 Inferring Balancing Selection From Genome-Scale Data. *Genome Biology and Evolution* 15:evad032.

Blighe, K., S. Rana, and M. Lewis, 2024 EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling. R package v.1.22.0 (https://github.com/kevinblighe/EnhancedVolcano).

Bourguet, D., 1999 The evolution of dominance. *Heredity* 83:1-4.

Bourguet, D., A. Genissel, and M. Raymond, 2000 Insecticide resistance and dominance levels. *Journal of Economic Entomology* 93:1588-1595.

Bourguet, D., T. Lenormand, T. Guillemaud, V. Marcel, D. Fournier et al., 1997 Variation of dominance of newly arisen adaptive genes. *Genetics* 147:1225-1234.

Brisson, D., 2018 Negative Frequency-Dependent Selection Is Frequently Confounding. *Frontiers in Ecology and Evolution* 6:10.

Brown, K. E., and J. K. Kelly, 2017 Antagonistic pleiotropy can maintain fitness variation in annual plants. *Journal of Evolutionary Biology* 31:46-56.

Brud, E., 2025 Season-specific dominance broadly stabilizes polymorphism under symmetric and asymmetric multivoltinism. *Genetics* 229:iyaf028

Buenrostro, J. D., P. G. Giresi, L. C. Zaba, H. Y. Chang and W. J. Greenleaf, 2013 Transposition of native chromatin for fast and sensitive epigenomic profiling of open

chromatin, DNA-binding proteins and nucleosome position. *Nature Methods* 10: 1213-1218.

Buenrostro, J. D., B. Wu, H. Y. Chang and W. J. Greenleaf, 2015 ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Current Protocols in Molecular Biology* 109: 21.29.21-21.29.29.

Charlesworth, B., 2015 Causes of natural variation in fitness: evidence from studies of Drosophila populations. *Proceedings of the National Academy of Sciences USA* 112: 1662-1669.

Charlesworth, B., and D. Charlesworth, 2010 *Elements of Evolutionary Genetics*. Roberts and Company Publishers, Greenwood Village, Colorado.

Charlesworth, B., and D. Charlesworth, 2017 Population genetics from 1966 to 2016. *Heredity* 118:2-9.

Charlesworth, B., and K. A. Hughes, 2000 The Maintenance of Genetic Variation in Life-History Traits, pp. 369-392 in *Evolutionary Genetics - From Molecules to Morphology*, edited by R. S. Singh and C. B. Krimbas. Cambridge University Press, Cambridge.

Chen, J., V. Nolte and C. Schlötterer, 2015 Temperature Stress Mediates Decanalization and Dominance of Gene Expression in *Drosophila melanogaster. PLoS Genetics* 11:e1004883.

Chevin, L.-M., Z. Gompert and P. Nosil, 2022 Frequency dependence and the predictability of evolution in a changing environment. *Evolution Letters* 6: 21-33.

Christie, M. R., and G. G. McNickle, 2023 Negative frequency dependent selection unites ecology and evolution. *Ecology and Evolution* 13:e10327.

Connallon, T., and S. F. Chenoweth, 2019 Dominance reversals and the maintenance of genetic variation for fitness. *PLoS Biology* 17:e3000118.

Connallon, T., and A. G. Clark, 2012 A General Population Genetic Framework for Antagonistic Selection That Accounts for Demography and Recurrent Mutation. *Genetics* 190:1477-1489.

Connallon, T., and A. G. Clark, 2013 Antagonistic versus nonantagonistic models of balancing selection: characterizing the relative timescales and hitchhiking effects of partial selective sweeps. *Evolution* 67: 908-917.

Connallon, T., and A. G. Clark, 2014 Balancing Selection in Species with Separate Sexes: Insights from Fisher's Geometric Model. *Genetics* 197:991-1006.

Corbett-Detig, R.B., C. Cardeno, and C.H. Langley, 2012. Sequence-based detection and breakpoint assembly of polymorphic inversions. *Genetics* 192:131-137.

Corces, M. R., A. E. Trevino, E. G. Hamilton, P. G. Greenside, N. A. Sinnott-Armstrong et al., 2017 An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nature Methods 14:959-962.

Crow, J., 1970 Genetic loads and the cost of natural selection, pp. 128-177 in *Mathematical Topics in Population Genetics*, edited by K.-I. Kojima. Springer, Berlin,

Crow, T., J. Ta, S. Nojoomi, M. R. Aguilar-Rangel, J. V. Torres Rodríguez et al., 2020 Gene regulatory effects of a large chromosomal inversion in highland maize. *PLoS Genetics* 16: e1009213.

Croze, M., A. Wollstein, V. Božičević, D. Živković, W. Stephan et al., 2017 A genome-wide scan for genes under balancing selection in *Drosophila melanogaster. BMC Evolutionary Biology* 17: 15.

Croze, M., D. Živković, W. Stephan and S. Hutter, 2016 Balancing selection on immunity genes: review of the current literature and new analysis in *Drosophila melanogaster. Zoology* 119: 322-329.

Curtsinger, J. W., P. M. Service and T. Prout, 1994 Antagonistic pleiotropy, reversal of dominance, and genetic polymorphism. *American Naturalist* 144: 210-228.

Danecek, P., J. K. Bonfield, J. Liddle, J. Marshall, V. Ohan et al., 2021 Twelve years of SAMtools and BCFtools. *Gigascience* 10(2):giab008.

Davies, A. G., A. Y. Game, Z. Chen, T. J. Williams, S. Goodall et al., 1996 S*calloped wings* is the *Lucilia cuprina Notch* homologue and a candidate for the modifier of fitness and asymmetry of diazinon resistance. *Genetics* 143:1321-1337.

Day, A. W., and J. E. Cummins, 1975 Evidence for a new kind of regulatory gene controlling expression of genes for morphogenesis during the cell cycle in *Ustilago violacea. Genetical Research* 25:253-266.

Dempster, E. R., 1955 Maintenance of genetic heterogeneity, pp. 25-32 in *Cold Spring Harbor Symposia on Quantitative Biology*. Cold Spring Harbor Laboratory Press.

de Wit, E., F. Greil and B. van Steensel, 2007 High-resolution mapping reveals links of HP1 with active and inactive chromatin components. *PLoS Genetics* 3: e38.

Di, C., and K. E. Lohmueller, 2024 Revisiting Dominance in Population Genetics. *Genome Biology and Evolution* 16:evae147.

Di Mauro, G., A. Carbonell, P. Escudero-Ferruz and F. Azorín, 2020 The zinc-finger proteins WOC and ROW play distinct functions within the HP1c transcription complex. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms* 1863: 194492.

Doebley, J., A. Stec and C. Gustus, 1995 *teosinte branched1* and the origin of maize: evidence for epistasis and the evolution of dominance. *Genetics* 141:333-346.

Dolgin, E. S., and S. P. Otto, 2003 Segregation and the evolution of sex under overdominant selection. *Genetics* 164:1119-1128.

Duchemin, W., P.-Y. Dupont, M. A. Campbell, A. R. Ganley and M. P. Cox, 2015 HyLiTE: accurate and flexible analysis of gene expression in hybrid and allopolyploid species. *BMC Bioinformatics* 16:8.

Durand, E., R. Méheust, M. Soucaze, P. M. Goubet, S. Gallina et al., 2014 Dominance hierarchy arising from the evolution of a complex small RNA regulatory network. *Science* 346:1200-1205.

Durmaz, E., C. Benson, M. Kapun, P. Schmidt and T. Flatt, 2018 An inversion supergene in *Drosophila* underpins latitudinal clines in survival traits. J*ournal of Evolutionary Biology* 31:1354-1364.

Falconer, D. S., and T. F. C. Mackay, 1996 *Introduction to Quantitative Genetics.* Longman, Essex, England.

Fijarczyk, A., and W. Babik, 2015 Detecting balancing selection in genomes: limits and prospects. *Molecular Ecology* 24:3529-3545.

Fisher, R. A., 1928 The Possible Modification of the Response of the Wild Type to Recurrent Mutations*. American Naturalist* 62:115-126.

Fisher, R. A., 1930a *The Genetical Theory of Natural Selection*. Oxford at the Clarendon Press, Oxford.

Fisher, R. A., 1930b The Evolution of Dominance in Certain Polymorphic Species. *The American Naturalist* 64: 385-406.

Fisher, R. A., 1938 Dominance in poultry feathered feet, rose comb, internal pigment and pile. *Proceedings of the Royal Society of London B* 125:25-48.

Flatt, T., 2020 Life-History Evolution and the Genetics of Fitness Components in *Drosophila melanogaster. Genetics* 214: 3-48.

Flintham, E., V. Savolainen, S. P. Otto, M. Reuter, and C. Mullon, 2025 The maintenance of genetic polymorphism underlying sexually antagonistic traits. *Evolution Letters* 9:259-272.

Font-Burgada, J., D. Rossell, H. Auer, and F. Azorín, 2008 *Drosophila* HP1c isoform interacts with the zinc-finger proteins WOC and Relative-of-WOC to regulate gene expression. *Genes & Development* 22:3007-3023.

Fuller, Z. L., G. D. Haynes, S. Richards, and S. W. Schaeffer, 2016 Genomics of Natural Populations: How Differentially Expressed Genes Shape the Evolution of Chromosomal Inversions in *Drosophila pseudoobscura. Genetics* 204:287-301.

Fyon, F., A. Cailleau, and T. Lenormand, 2015 Enhancer Runaway and the Evolution of Diploid Gene Expression. *PLOS Genetics* 11:e1005665.

Ge, S. X., D. Jung, and R. Yao, 2020 ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics* 36:2628-2629.

Gemmell, N. J., and J. Slate, 2006 Heterozygote Advantage for Fecundity. PLoS ONE 1:e125.

Ghavi-Helm, Y., 2020 Functional Consequences of Chromosomal Rearrangements on Gene Expression: Not So Deleterious After All? *Journal of Molecular Biology* 432: 665-675.

Gibson, G., R. Riley-Berger, L. Harshman, A. Kopp, S. Vacha et al., 2004 Extensive sex-specific nonadditivity of gene expression in Drosophila melanogaster. Genetics 167: 1791-1799.

Gilchrist, M. A., and H. F. Nijhout, 2001 Nonlinear developmental processes as sources of dominance. *Genetics* 159:423-432.

Gillespie, J. H., 1978 A general model to account for enzyme variation in natural populations. V. The SAS-CFF model. *Theoretical Population Biology* 14:1-45.

Gillespie, J. H., and C. H. Langley, 1974 A general model to account for enzyme variation in natural populations. *Genetics* 76: 837-848.

Giner-Delgado, C., S. Villatoro, J. Lerga-Jaso, M. Gayà-Vidal, M. Oliva et al., 2019 Evolutionary and functional impact of common polymorphic inversions in the human genome. *Nature Communications* 10:4222.

Glaser-Schmitt, A., M. J. Wittmann, T. J. S. Ramnarine and J. Parsch, 2021 Sexual Antagonism, Temporally Fluctuating Selection, and Variable Dominance Affect a Regulatory Polymorphism in *Drosophila melanogaster. Molecular Biology and Evolution* 38:4891-4907.

Glaser-Schmitt, A., M. Lemoine, M. Kaltenpoth, and J. Parsch, 2024 Pervasive tissue-, genetic background-, and allele-specific gene expression effects in *Drosophila melanogaster. PLOS Genetics* 20:e1011257.

Grant, C. E., and T. L. Bailey, 2021 XSTREME: Comprehensive motif analysis of biological sequence datasets. *bioRxiv*: 10.1101/2021.09.02.458722

Grieshop, K., and G. Arnqvist, 2018 Sex-specific dominance reversal of genetic variation for fitness. *PLoS Biology* 16 e2006810.

Grieshop, K., E. K. H. Ho and K. R. Kasimatis, 2024 Dominance reversals: the resolution of genetic conflict and maintenance of genetic variation. P*roceedings of the Royal Society B* 291: 20232816.

Gruber, J. D., K. Vogel, G. Kalay and P. J. Wittkopp, 2012 Contrasting properties of gene-specific regulatory, coding, and copy number mutations in Saccharomyces cerevisiae: frequency, effects, and dominance. *PLoS Genetics* 8:e1002497.

Gutiérrez-Valencia, J., P. W. Hughes, E. L. Berdan and T. Slotte, 2021 The genomic architecture and evolutionary fates of supergenes. *Genome Biology and Evolution* 13:evab057.

Guyot, L., T. Giraud, and P. Jay, 2025 Loci under balancing selection facilitate the emergence of pseudo-overdominance and recombination suppression. *bioRxiv*:2025.2006.2019.660549.

Haldane, J. B. S., 1930 A Note on Fisher's Theory of the Origin of Dominance, and on a Correlation between Dominance and Linkage. *American Naturalist* 64:87-90.

Haldane, J. B. S., 1948 The theory of a cline. *Journal of Genetics* 48:277-284.

Haldane, J. B. S., and S. D. Jayakar, 1963 Polymorphism due to selection of varying direction. *Journal of Genetics* 58: 237-242.

Hedrick, P. W., 1976 Genetic variation in a heterogeneous environment. II. Temporal heterogeneity and directional selection. *Genetics* 84:145-157.

Hedrick, P. W., 2011 Population genetics of malaria resistance in humans. *Heredity* 107:283-304.

Hedrick, P. W., 2012 What is the evidence for heterozygote advantage selection? *Trends in Ecology and Evolution* 27:698-704.

Hofbauer, L., L.-M. Pleyer, F. Reiter, A. Schleiffer, A. Vlasova, L. Serebreni, A. Huang and A. Stark, 2024 A genome-wide screen identifies silencers with distinct chromatin properties and mechanisms of repression. *Molecular Cell* 84:4503-4521.e4514.

Hoffmann, A. A., and T. Flatt, 2022 The rapid tempo of adaptation. *Science* 375: 1226-1227.

Houle, D., 1992 Comparing Evolvability and Variability of Quantitative Traits. *Genetics* 130:195-204.

Huber, C. D., A. Durvasula, A. M. Hancock and K. E. Lohmueller, 2018 Gene expression drives the evolution of dominance. *Nature Communications* 9:2750.

Hughes, K. A., J. F. Ayroles, M. M. Reedy, J. M. Drnevich, K. C. Rowe et al., 2006 Segregating variation in the transcriptome: cis regulation and additivity of effects. *Genetics* 173:1347-1355.

Hurst, L. D., and J. P. Randerson, 2000 Dosage, Deletions and Dominance: Simple Models of the Evolution of Gene Expression. *Journal of Theoretical Biology* 205:641-647.

Johnson, O. L., R. Tobler, J. M. Schmidt, and C. D. Huber, 2023 Fluctuating selection and the determinants of genetic variation. *Trends in Genetics* 39:491-504.

Johnson, T., and N. Barton, 2005 Theoretical models of selection and mutation on quantitative traits. *Philosophical Transactions of the Royal Society B* 360:1411-1425.

Johnston, S. E., J. Gratten, C. Berenos, J. G. Pilkington, T. H. Clutton-Brock, J. M. Pemberton, and J. Slate 2013 Life history trade-offs at a single locus maintain sexually selected genetic variation. *Nature* 502:93-95.

Kacser, H., and J. A. Burns, 1981 The molecular basis of dominance. *Genetics* 97:639-666.

Kapun, M., and T. Flatt, 2019 The adaptive significance of chromosomal inversion polymorphisms in *Drosophila melanogaster. Molecular Ecology* 28:1263-1282.

Kapun, M., D. K. Fabian, J. Goudet and T. Flatt, 2016a Genomic Evidence for Adaptive Inversion Clines in *Drosophila melanogaster. Molecular Biology and Evolution* 33:1317-1336.

Kapun, M., E. Durmaz Mitchell, T. J. Kawecki, P. Schmidt and T. Flatt, 2023 An Ancestral Balanced Inversion Polymorphism Confers Global Adaptation. *Molecular Biology and Evolution* 40:msad118.

Kapun, M., C. Schmidt, E. Durmaz, P. S. Schmidt and T. Flatt, 2016b Parallel effects of the inversion *In(3R)Payne* on body size across the North American and Australian clines in *Drosophila melanogaster*. *Journal of Evolutionary Biology* 29:1059-1072.

Karageorgi, M., A. S. Lyulina, M. C. Bitter, E. Lappo, S. I. Greenblum, Z. K. Mouza, C. T. Tran, A. V. Huynh, H. Oken, P. Schmidt, and D. Petrov, 2024 Dominance reversal protects large-effect resistance polymorphisms in temporally varying environments. *bioRxiv*: 2024.2010.2023.619953.

Kaufmann, P., J.L. Rönn, E. Immonen, and G. Arnqvist, 2024 Sex-Specific Dominance of Gene Expression in Seed Beetles. *Molecular Biology and Evolution* 41:msae244.

Kavi, H. H., and J. A. Birchler, 2009 *Drosophila* KDM2 is a H3K4me3 demethylase regulating nucleolar organization. *BMC Research Notes* 2:217.

Kay, M., 2024 ggdist: Visualizations of Distributions and Uncertainty in the Grammar of Graphics. *IEEE Transactions on Visualization and Computer Graphics* 30:414-424.

Keightley, P. D., 1996 Metabolic Models of Selection Response. *Journal of Theoretical Biology* 182:311-316.

Kennington, W. J., A. A. Hoffmann, and L. Partridge, 2007 Mapping regions within cosmopolitan inversion *In(3R)Payne* associated with natural variation in body size in *Drosophila melanogaster. Genetics* 177:549-556.

Kessler, R., J. Tisserand, J. Font-Burgada, O. Reina, L. Coch et al., 2015 dDsk2 regulates H2Bub1 and RNA polymerase II pausing at dHP1c complex target genes. *Nature Communications* 6: 7049.

Khudiakova, K.A., N.H. Barton, and G. Arnqvist, 2025 Sign epistasis extends the effects of balancing selection on genetic diversity. *bioRxiv*:2025.2004.2009.647826.

Kidwell, J. F., M. T. Clegg, F. M. Stewart and T. Prout, 1977 Regions of stable equilibria for models of differential selection in the two sexes under random mating. *Genetics* 85:171-183.

Kim, Y., 2023 Partial protection from fluctuating selection leads to evolution towards wider population size fluctuation and a novel mechanism of balancing selection. *Proceedings of the Royal Society B* 290:20230822.

Kwon, S. H., and J. L. Workman, 2011 HP1c casts light on dark matter. *Cell Cycle* 10:625-630.

Kwon, S. H., L. Florens, S. K. Swanson, M. P. Washburn, S. M. Abmayr et al., 2010 Heterochromatin protein 1 (HP1) connects the FACT histone chaperone complex to the phosphorylated CTD of RNA polymerase II. *Genes & Development* 24:2133-2145.

Lagarou, A., A. Mohd-Sarip, Y. M. Moshkin, G. E. Chalkley, K. Bezstarosti, J. A. A. Demmers, and C. P. Verrijzer, 2008 dKDM2 couples histone H2A ubiquitylation to histone H3 demethylation during Polycomb group silencing. *Genes & Development* 22:2799-2810.

Lagervall, P. M., 1961 Quantitative inheritance and dominance. IV. The average degree of dominance. *Hereditas* 47:197-202.

Lande, R., 1975 The maintenance of genetic variability by mutation in a polygenic character with linked loci. *Genetical Research* 26:221-235.

Langmead, B., and S. L. Salzberg, 2012 Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9:357-359.

Lavington, E., and A. D. Kern, 2017 The Effect of Common Inversion Polymorphisms *In(2L)t* and *In(3R)Mo* on Patterns of Transcriptional Variation in *Drosophila melanogaster. G3* 7:3659-3668.

Lemeunier F., and S. Aulard, 1992 Inversion polymorphism in *Drosophila melanogaster*, pp. 339–405 in *Drosophila Inversion Polymorphism*, edited by C.B. Krimbas and J.R. Powell. CRC Press, Boca Raton, Florida.

Lemos, B., L. O. Araripe, P. Fontanillas and D. L. Hartl, 2008 Dominance and the evolutionary accumulation of cis- and trans-effects on gene expression. *Proceedings of the National Academy of Sciences* 105:14471-14476.

Le Poul, Y., A. Whibley, M. Chouteau, F. Prunier, V. Llaurens et al., 2014 Evolution of dominance mechanisms at a butterfly mimicry supergene. *Nature Communications* 5:5644.

Levene, H., 1953 Genetic equilibrium when more than one ecological niche is available. *American Naturalist* 87:331-333.

Lewontin, R. C., 1958 A general method for investigating the equilibrium of gene frequency in a population. *Genetics* 43:419-434.

Lewontin, R. C., 1974 *The Genetic Basis of Evolutionary Change*. Columbia University Press, New York.

Li, J., and C. Bank, 2024 Dominance and multi-locus interaction. *Trends in Genetics* 40:364-378.

Llaurens, V., M. Joron and S. Billiard, 2015 Molecular mechanisms of dominance evolution in Müllerian mimicry. *Evolution* 69:3097-3108.

Lopez-Delisle, L., L. Rabbani, J. Wolff, V. Bhardwaj, R. Backofen et al., 2021 pyGenomeTracks: reproducible plots for multivariate genomic datasets. *Bioinformatics* 37:422-423.

Love, M. I., W. Huber and S. Anders, 2014 Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15:550.

Lynch, M., and B. Walsh, 1998 *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, Mass.

Machado, H. E., A. O. Bergland, R. Taylor, S. Tilk, E. Behrman et al., 2021 Broad geographic sampling reveals the shared basis and environmental correlates of seasonal adaptation in *Drosophila. eLife* 10:e67577.

Manna, F., G. Martin, and T. Lenormand, 2011 Fitness Landscapes: An Alternative Theory for the Dominance of Mutation. *Genetics* 189:923-937.

Matsui, T., M. N. Mullis, K. R. Roy, J. J. Hale, R. Schell, et al., 2022 The interplay of additivity, dominance, and epistasis on fitness in a diploid yeast cross. *Nature Communications* 13:1463.

Matzkin, L. M., T. J. S. Merritt, C.-T. Zhu, and W. F. Eanes, 2005 The structure and population genetics of the breakpoints associated with the cosmopolitan chromosomal inversion *In(3R)Payne* in *Drosophila melanogaster*. *Genetics* 170:1143-1152.

Mayo, O., and R. Bürger, 1997 The evolution of dominance: a theory whose time has passed? *Biological Reviews* 72:97-110.

McKenzie, J. A., and A. Y. Game, 1987 Diazinon resistance in *Lucilia cuprina*; mapping of a fitness modifier. *Heredity* 59:371-381.

McManus, C. J., J. D. Coolon, M. O. Duff, J. Eipper-Mains, B. R. Graveley, et al., 2010 Regulatory divergence in *Drosophila* revealed by mRNA-seq. *Genome Research* 20: 816-825.

Meiklejohn, C. D., J. D. Coolon, D. L. Hartl, and P. J. Wittkopp, 2014 The roles of cis- and trans-regulation in the evolution of regulatory incompatibilities and sexually dimorphic gene expression. *Genome Research* 24:84-95.

Mérot, C., V. Llaurens, E. Normandeau, L. Bernatchez, and M. Wellenreuther, 2020 Balancing selection via life-history trade-offs maintains an inversion polymorphism in a seaweed fly. *Nature Communications* 11:670.

Moorman, C., L. V. Sun, J. Wang, E. de Wit, W. Talhout, et al., 2006 Hotspots of transcription factor colocalization in the genome of *Drosophila melanogaster. Proceedings of the National Academy of Sciences* 103:12027-12032.

Moretti, J., P. Chastagner, C. C. Liang, M. A. Cohn, A. Israël, et al., 2012 The ubiquitin-specific protease 12 (USP12) is a negative regulator of notch signaling acting on notch receptor trafficking toward degradation. *Journal of Biological Chemistry* 287:29429-29441.

Mishra, P., T. S. Barrera, K. Grieshop, and A. F. Agrawal, 2024 Cis-regulatory variation in relation to sex and sexual dimorphism in *Drosophila melanogaster. Genome Biology and Evolution* 16:evae234.

Müller, K., and H. Wickham, 2023 tibble: Simple Data Frames (https://tibble.tidyverse.org/, https://github.com/tidyverse/tibble).

Naisbit, R. E., C. D. Jiggins, and J. Mallet, 2003 Mimicry: developmental genes that contribute to speciation. Evolution & Development 5: 269-280.

Nassar, R., H. J. Muhs, and R. D. Cook, 1973 Frequency-dependent selection at the *Payne* inversion in *Drosophila melanogaster*. *Evolution* 27:558-564.

Neuwirth, E., 2022 RColorBrewer: ColorBrewer palettes. (https://CRAN.R-project.org/package=RColorBrewer).

Ohh, B. K., and B. L. Sheldon, 1970 Selection for dominance of *hairy-wing* (*Hw*) in *Drosophila melanogaster* I. Dominance at different levels of phenotype. *Genetics* 66: 517-540.

Omholt, S. W., E. Plahte, L. Oyehaug, and K. Xiang, 2000 Gene Regulatory Networks Generating the Phenomena of Additivity, Dominance and Epistasis. *Genetics* 155:969-980.

Orr, H. A., 1991 A test of Fisher's theory of dominance. *Proceedings of the National Academy of Sciences* 88:11413-11415.

Osada, N., R. Miyagi, and A. Takahashi, 2017 Cis- and Trans-regulatory Effects on Gene Expression in a Natural Population of *Drosophila melanogaster. Genetics* 206:2139-2148.

Otto, S. P., and D. Bourguet, 1999 Balanced Polymorphisms and the Evolution of Dominance. *American Naturalist* 153:561-574.

Paris, M., F. Roux, A. Bérard, and X. Reboud, 2008 The effects of the genetic background on herbicide resistance fitness cost and its associated dominance in *Arabidopsis thaliana. Heredity* 101:499-506.

Paris, M., E. Durmaz Mitchell, E. Kerdaffrec, D. Rubin, C. Spichtig, F. Zurbriggen, J. Becker, H. Augustijnen, H. Thyagarajan, E. Zinn, et al. 2025 Multiple Forms of Balancing Selection Maintain Inversion Polymorphism. *Heredity*, in press.

Parsons, P.A., and W. F. Bodmer, 1961. The Evolution of Overdominance: Natural Selection and Heterozygote Advantage. *Nature* 190:7-12.

Patro, R., G. Duggal, M. I. Love, R. A. Irizarry, and C. Kingsford, 2017 Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods* 14:417-419.

Patten, M. M., D. Haig, and F. Úbeda, 2010 Fitness variation due to sexual antagonism and linkage disequilibrium. *Evolution* 64:3638-3642.

Pearse, D. E., N. J. Barson, T. Nome, G. Gao, M. A. Campbell, et al., 2019 Sex-dependent dominance maintains migration supergene in rainbow trout. *Nature Ecology & Evolution* 3:1731-1742.

Pei, Y., W. Forstmeier, U. Knief, and B. Kempenaers, 2023 Weak antagonistic fitness effects can maintain an inversion polymorphism. *Molecular Ecology* 32: 3575-3585.

Plutynski, A., 2008 Explaining how and explaining why: developmental and evolutionary explanations of dominance. *Biology & Philosophy* 23:363-381.

Porter, A. H., N. A. Johnson, and A. Y. Tulchinsky, 2017 A New Mechanism for Mendelian Dominance in Regulatory Genetic Pathways: Competitive Binding by Transcription Factors. *Genetics* 205:101-112.

Porter, A. H., N. A. Johnson, and A. Y. Tulchinsky, 2025. Fitness landscapes of simple regulatory genetic interactions show pervasive heterozygote advantage and support stable polymorphism. *Journal of Theoretical Biology* 112233, in press.

Posavi, M., G. W. Gelembiuk, B. Larget, and C. E. Lee, 2014 Testing for beneficial reversal of dominance during salinity shifts in the invasive copepod *Eurytemora affinis*, and implications for the maintenance of genetic variation. *Evolution* 68:3166-3183.

Proulx, S. R., and H. Teotónio, 2022 Selection on modifiers of genetic architecture under migration load. *PLoS Genetics* 18:e1010350.

Prout, T., 2000 How well does opposing selection maintain variation? pp. 157-181 in *Evolutionary Genetics - From Molecules to Morphology,* edited by R. S. Singh and C. B. Krimbas. Cambridge University Press, Cambridge.

Puixeu, G., A. Macon, and B. Vicoso, 2023. Sex-specific estimation of cis and trans regulation of gene expression in heads and gonads of *Drosophila melanogaster*. *G3* 13:jkad121.

Quinlan, A. R., and I. M. Hall, 2010 BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26:841-842.

Rako, L., A. R. Anderson, C. M. Sgro, A. J. Stocker and A. A. Hoffmann, 2006 The association between inversion *In(3R)Payne* and clinally varying traits in *Drosophila melanogaster. Genetica* 128: 373-384.

Ramírez, F., V. Bhardwaj, L. Arrigoni, K. C. Lam, B. A. Grüning et al., 2018 High-resolution TADs reveal DNA sequences underlying genome organization in flies. *Nature Communications* 9:189.

Ramírez, F., D. P. Ryan, B. Grüning, V. Bhardwaj, F. Kilpert et al., 2016 deepTools2: a next generation web server for deep-sequencing data analysis. *Nucleic Acids Research* 44:W160-165.

Reid, J. M., 2022 Intrinsic emergence and modulation of sex-specific dominance reversals in threshold traits. *Evolution* 76:1924-1941.

Rockman, M. V. and L. Kruglyak, 2006. Genetics of global gene expression. N*ature Reviews Genetics* 7:862–872.

Rose, M. R., 1982 Antagonistic pleiotropy, dominance, and genetic variation. *Heredity* 48:63-78.

Rose, M. R., 1985 Life history evolution with antagonistic pleiotropy and overlapping generations. *Theoretical Population Biology* 28:342-358.

Roux, F., C. Camilleri, S. Giancola, D. Brunel and X. Reboud, 2005 Epistatic interactions among herbicide resistances in *Arabidopsis thaliana*: the fitness cost of multiresistance. *Genetics* 171:1277-1288.

Rudman, S. M., S. I. Greenblum, S. Rajpurohit, N. J. Betancourt, J. Hanna et al., 2022 Direct observation of adaptive tracking on ecological time scales in *Drosophila. Science* 375:eabj7484.

Rusuwa, B. B., H. Chung, S. L. Allen, F. D. Frentiu and S. F. Chenoweth, 2022 Natural variation at a single gene generates sexual antagonism across fitness components in *Drosophila. Current Biology* 32:3161-3169.e3167.

Ruzicka, F., M.K. Zwoinska, D. Goedert, H. Kokko, X.-Y.L. Richter, I.R. Moodie, S. Nilén, C. Olito, E. I. Svensson, P. Czuppon, et al., 2025 A century of theories of balancing selection. *bioRxiv*:2025.2002.2012.637871.

Said, I., A. Byrne, V. Serrano, C. Cardeno, C. Vollmers et al., 2018 Linked genetic variation and not genome structure causes widespread differential expression associated with chromosomal inversions. *Proceedings of the National Academy of Sciences USA* 115:5492-5497.

Schaal, S. M., B. C. Haller and K. E. Lotterhos, 2022 Inversion invasions: when the genetic basis of local adaptation is concentrated within inversions in the face of gene flow. *Philosophical Transactions of the Royal Society B* 377:20210200.

Schoelz, J. M., J. X. Feng and N. C. Riddle, 2021 The *Drosophila* HP1 family is associated with active gene expression across chromatin contexts. *Genetics* 219:iyab108.

Schoelz, J. M., and N. C. Riddle, 2022 Functions of HP1 proteins in transcriptional regulation. *Epigenetics & Chromatin* 15:14.

Schwander, T., Libbrecht, R., and L. Keller, 2014 Supergenes and complex phenotypes. *Current Biology* 24:R288–R294.

Sharp, N. P., and A. F. Agrawal, 2018 An experimental test of the mutation-selection balance model for the maintenance of genetic variance in fitness components. *Proceedings of the Royal Society B* 285: 20181864.

Shi, X., D. W. K. Ng, C. Zhang, L. Comai, W. Ye, Z. J. Chen, 2012 Cis- and trans-regulatory divergence between progenitor species determines gene-expression novelty in *Arabidopsis* allopolyploids. *Nature Communications* 3:950.

Signor, S. A. and S. V. Nuzhdin, 2018. The Evolution of Gene Expression in cis and trans. *Trends in Genetics* 34:532-544.

Siljestam, M., C. Rueffler and G. Arnqvist, 2024 Sex-Specific Dominance and Its Effects on Allelic Diversity in Sexually Antagonistic Loci. *bioRxiv:*2024.2005.2006.592709.

Soneson, C., M. I. Love, and M. D. Robinson, 2015 Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000 Research* 4:1521.

Spencer, H. G., and N. K. Priest, 2016 The Evolution of Sex-Specific Dominance in Response to Sexually Antagonistic Selection. *American Naturalist* 187:658-666.

Spencer, Z. T., V. H. Ng, H. Benchabane, G. S. Siddiqui, D. Duwadi et al., 2023 The USP46 deubiquitylase complex increases Wingless/Wnt signaling strength by stabilizing Arrow/LRP6. *Nature Communications* 14:6174.

Stephan, W., and A. C. Hörger, 2019 *Molekulare Populationsgenetik: Theoretische Konzepte und empirische Evidenz*. Springer, Berlin.

Sun, D., I. Huh, W. M. Zinzow-Kramer, D. L. Maney and S. V. Yi, 2018 Rapid regulatory evolution of a nonrecombining autosome linked to divergent behavioral phenotypes. *Proceedings of the National Academy of Sciences USA* 115:2794-2799.

Sun, J., X. Wang, R. G. Xu, D. Mao, D. Shen et al., 2021 HP1c regulates development and gut homeostasis by suppressing Notch signaling through Su(H). *EMBO Reports* 22:e51298.

Sved, J. A., and O. Mayo, 1970 The Evolution of Dominance, pp. 289-316 in *Mathematical Topics in Population Genetics*, edited by K.-i. Kojima. Springer Berlin Heidelberg, Berlin, Heidelberg.

Takahashi, A., and T. Takano-Shimizu, 2011 Divergent enhancer haplotype of ebony on inversion *In(3R)Payne* associated with pigmentation variation in a tropical population of *Drosophila melanogaster. Molecular Ecology,* 20:4277-4287.

Tarutani, Y., H. Shiba, M. Iwano, T. Kakizaki, G. Suzuki et al., 2010 Trans-acting small RNA determines dominance relationships in *Brassica* self-incompatibility. Nature 466:983-986.

Thompson, J. N., and J. M. Thoday, 1972 Modification of dominance by selection in the homozygote. *Heredity* 29:285-292.

Thompson, M. J., and C. D. Jiggins, 2014 Supergenes and their role in evolution. *Heredity* 113:1-8.

Turelli, M., and N. H. Barton, 2004 Polygenic variation maintained by balancing selection: pleiotropy, sex-dependent allelic effects and G x E interactions. *Genetics* 166:1053-1079.

Van Dooren, T. J. M., 1999 The Evolutionary Ecology of Dominance-Recessivity. *Journal of Theoretical Biology* 198:519-532.

Van Dooren, T. J., 2006 Protected polymorphism and evolutionary stability in pleiotropic models with trait-specific dominance. *Evolution* 60:1991-2003.

Veitia, R. A., S. Bottani and J. A. Birchler, 2013 Gene dosage effects: nonlinearities, genetic interactions, and dosage compensation. *Trends in Genetics* 29:385-393

Wallace, B., 1968 *Topics in Population Genetics.* W. W. Norton & Co., New York.

Walsh, B. and M. W. Blows, 2009 Abundant genetic variation + strong selection = multivariate genetic constraints: A geometric view of adaptation. *Annual Review of Ecology, Evolution, and Systematics* 40:41-59.

Wang, L., J. W. Israel, A. Edgar, R. A. Raff, E. C. Raff, M. Byrne and G. A. Wray, 2020. Genetic basis for divergence in developmental gene expression in two closely related sea urchins. *Nature Ecology and Evolution* 4:831-840.

Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis.* Springer, New York.

Wickham, H., François, R., Henry, L., Müller, K., and D. Vaughan, 2023 dplyr: A Grammar of Data Manipulation. R package v. 1.1.4 (https://github.com/tidyverse/dplyr, https://dplyr.tidyverse.org)

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., and H. Yutani, 2019 Welcome to the tidyverse. *Journal of Open Source Software* 4(43):1686.

Wittkopp, P. J., B. K. Haerum and A. G. Clark, 2008. Regulatory changes underlying expression differences within and between *Drosophila* species. *Nature Genetics* 40:346-350.

Wittmann, M. J., A. O. Bergland, M. W. Feldman, P. S. Schmidt, and D. A. Petrov, 2017 Seasonally fluctuating selection can maintain polymorphism at many loci via segregation lift. *Proceedings of the National Academy of Sciences USA* 114:E9932-E9941.

Wittmann, M. J., S. Mousset, and J. Hermisson, 2023 Modeling the genetic footprint of fluctuating balancing selection: from the local to the genomic scale. *Genetics* 223:iyad022.

Wray, G. A., 2007 The evolutionary significance of cis-regulatory mutations. *Nature Reviews Genetics* 8:206-216.

Wright, S., 1929 Fisher's Theory of Dominance. *American Naturalist* 63: 274-279.

Wright, S., 1934 Physiological and Evolutionary Theories of Dominance. The *American Naturalist* 68: 24-53.

Wright, S. 1977. *Evolution and the Genetics of Populations*. Vol. 3. Chicago: University Chicago Press.

Xu, K., R. Kosoy, K. Shameer, S. Kumar, L. Liu, B. Readhead, G. M. Belbin, H.-C. Lee, R. Chen, and J.T. Dudley, 2019 Genome-wide analysis indicates association between heterozygote advantage and healthy aging in humans. *BMC Genetics* 20:52.

Zajitschek, F., and T. Connallon, 2018 Antagonistic pleiotropy in species with separate sexes, and the maintenance of genetic variation in life-history traits and fitness. *Evolution* 72:1306-1316.

Zhang, X., A. J. Cal and J. O. Borevitz, 2011 Genetic architecture of regulatory variation in *Arabidopsis thaliana*. *Genome Research* 21:725-733.

Zhang, Y., T. Liu, C. A. Meyer, J. Eeckhoute, D. S. Johnson et al., 2008 Model-based Analysis of ChIP-Seq (MACS). *Genome Biology* 9:R137.

Zhu, A., J. G. Ibrahim and M. I. Love, 2019 Heavy-tailed prior distributions for sequence count data: removing the noise and preserving large differences. *Bioinformatics* 35:2084-2092.

Zschocke, J., P. H. Byers and A. O. M. Wilkie, 2022 Gregor Mendel and the concepts of dominance and recessiveness. *Nature Reviews Genetics* 23:387-388.

Figure legends

Figure 1. The impact of *3RP* on differential gene expression in embryos. (A, C, E) Volcano plots depicting the negative $\log_{10}$ of p_{adj} plotted against the $\log_2$ of the fold change (after shrinkage with the apeglm method during DeSeq2 analysis) for each gene in each of the three pairwise comparisons between the three karyotypes (STD/STD vs. INV/INV; HET [=heterokaryotype] vs. STD/STD; and HET vs. INV/INV). Dotted lines indicate cut-offs of $p_{adj} \leq 0.001$ and two-fold/half expression level. Blue dots indicate expression change above the fold-change threshold, magenta dots significant expression change, and orange dots expression change above both thresholds. Labeled dots indicate genes located within *3RP* with an expression change above both thresholds. Note that few genes in STD/STD vs. INV/INV (panel A) show an expression change above both thresholds; those that do largely reside within *3RP*. Also, note that only eight genes show differential expression above either or both thresholds in the HET vs. INV/INV comparison. (B, D, F) Plotting of the same data (as in panels A, C, E), depicting additionally the chromosomal location of each gene. The -$\log_{10}$ p_{adj}-value is encoded in the size and hue of each data point as indicated in (B). The STD/STD vs. INV/INV comparison (B) shows a clear enrichment of DEG on *3R* and within *3RP*. DEG in the HET vs. STD/STD comparison (D) mostly exhibit lower expression in HET, which is also visible in the corresponding volcano plot (B) as a "trail" on the left-hand side. (G, H) Bar plots indicating the fraction of DEG ($p_{adj} \leq 0.001$) in each genomic region. Asterisks indicate significant differences based on χ^2 tests (* denotes $p \leq 0.1$; and ** $p \leq 0.01$) against the mean of *X*, *2L*, *2R*, *3L*, and other genomic regions (comprising chromosomes *4*, *Y*, and unmapped regions), or between regions on *3R*. Note that *3R* and *3RP* (and, in particular, the inversion breakpoints) contribute disproportionately to differential expression in STD/STD vs. INV/INV (G), whereas in the HET vs. STD/STD comparison (H), genes in the inversion body show the highest value but are only significantly different from *3R* excluding the breakpoints. Notably, the numbers of DEG in the HET vs. INV/INV comparison are too low to be plotted in this way. (I) Venn diagram showing the number of overlapping DEG ($p_{adj} < 0.001$) between the different karyotypes. About two thirds of DEG in STD/STD vs. INV/INV make up about half of DEG in the HET vs. STD comparison.

Figure 2. Differential gene expression in wing discs (for explanations see Fig. 1). (A, C, E) Volcano plots and (B, D, F) plots of the chromosomal location of each gene show a clear enrichment of DEG on *3R* and within *3RP*. Moreover, comparing STD vs. INV/INV and HET vs. INV/INV reveals almost identical expression profiles (A, E). (G, H) Bar plots indicating the

fraction of differentially expressed genes ($p_{adj} \leq 0.001$) in each genomic region. Asterisks indicate significant differences after χ^2 tests (*: $p \leq 0.1$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$) against the mean of *X*, *2L*, *2R*, *3L*, and other genomic regions, or between regions on *3R*. *3RP* contributes disproportionately to differential gene expression. Note that the numbers of DEG in the HET vs. STD/STD comparison are too low to be plotted in this way. (I) Venn diagram showing the number of overlapping DEG ($p_{adj} < 0.001$) between the different karyotypes. About 20% of DEG in STD/STD vs. INV/INV make up about half of DEG in the HET vs. INV/INV comparison.

Figure 3. Density plots of accessible chromatin regions in wing discs after peak calling with MACS2. The top half of each plot shows relative density of peaks, the bottom half shows absolute numbers. Note that chromatin is consistently less accessible in pericentromeric regions. (A) Peaks shared between all karyotypes show an even distribution throughout the genome. (B, C) Peaks that were uniquely called in INV/INV and STD/STD show the highest density on *3R*. (D) Peaks uniquely called in HET are enriched on the *X* chromosome. (E) No local enrichment is observed for peaks shared between HET and INV/INV, but (F) peaks shared between HET and STD/STD show the highest density on *3R*.

Figure 4. Quantification of the mode of inheritance (dominance coefficient *h*) for genes differentially expressed ($p_{adj} < 0.01$) between *3RP* karyotypes. (A) Bar plots showing the distribution of *h*-values for DEG in embryos and wing discs. Most DEG in embryos have a dominance coefficient of around 1 (indicating dominance of INV), while in wing discs there is an enrichment of DEG with a dominance coefficient around 0 (indicating recessivity of INV and hence dominance of STD). (B) A heatmap of z-scores of the different modes of inheritance depicting over- (dark blue) and underrepresentation (light blue) in the two datasets. Dominance of INV was strongly overrepresented in embryos, while additivity and full recessivity of INV were overrepresented in wing discs. Specifically, in embryos, INV was fully dominant for 42.55% and recessive only for 15.18% of DEG. In wing discs, by contrast, INV was fully recessive for 23.76% and dominant only for 14.92% of DEG. Partial dominance and, especially, recessivity showed a similar trend (11.11% in embryos vs. 7.18% in discs; 2.44% in embryos vs. 11.05% in discs, respectively). 23.76% of DEG in wing discs showed full additivity, whereas in embryos this figure was only 9.49%. An equally high proportion of DEG exhibited underdominance in both datasets (18.97% in embryos, 18.23% in wing discs), while overdominance was equally underrepresented (0.27% in embryos, 1.1% in wing discs).

Figure 5. Quantification of *cis* vs. *trans* influences on gene expression differences. Panels (A) - (D) show the relative expression between the parental STD/STD vs. INV/INV homokaryotypes along the *x*-axis plotted against the relative allelic expression of the STD vs. the INV allele in the STD/INV heterokaryotype along the *y*-axis for (A) embryos, considering all genes; (B) embryos, considering the inverted *3RP* region only; (C) wing discs, considering

all genes; and (D) wing discs, considering the inverted *3RP* region only. Only genes for which allelic expression information is available are shown. For each comparison, the Pearson's correlation coefficient *R* and the corresponding *p*-value is given for all genes as well as only for genes that show a significant difference in expression as well as allelic expression ($p < 0.05$). Green coloring indicates genes that are classified as only regulated by *cis* effects, orange indicates genes classified as only regulated by *trans* effects, and blue are genes in which *cis* and *trans* effects are co-acting. See main text for further details.

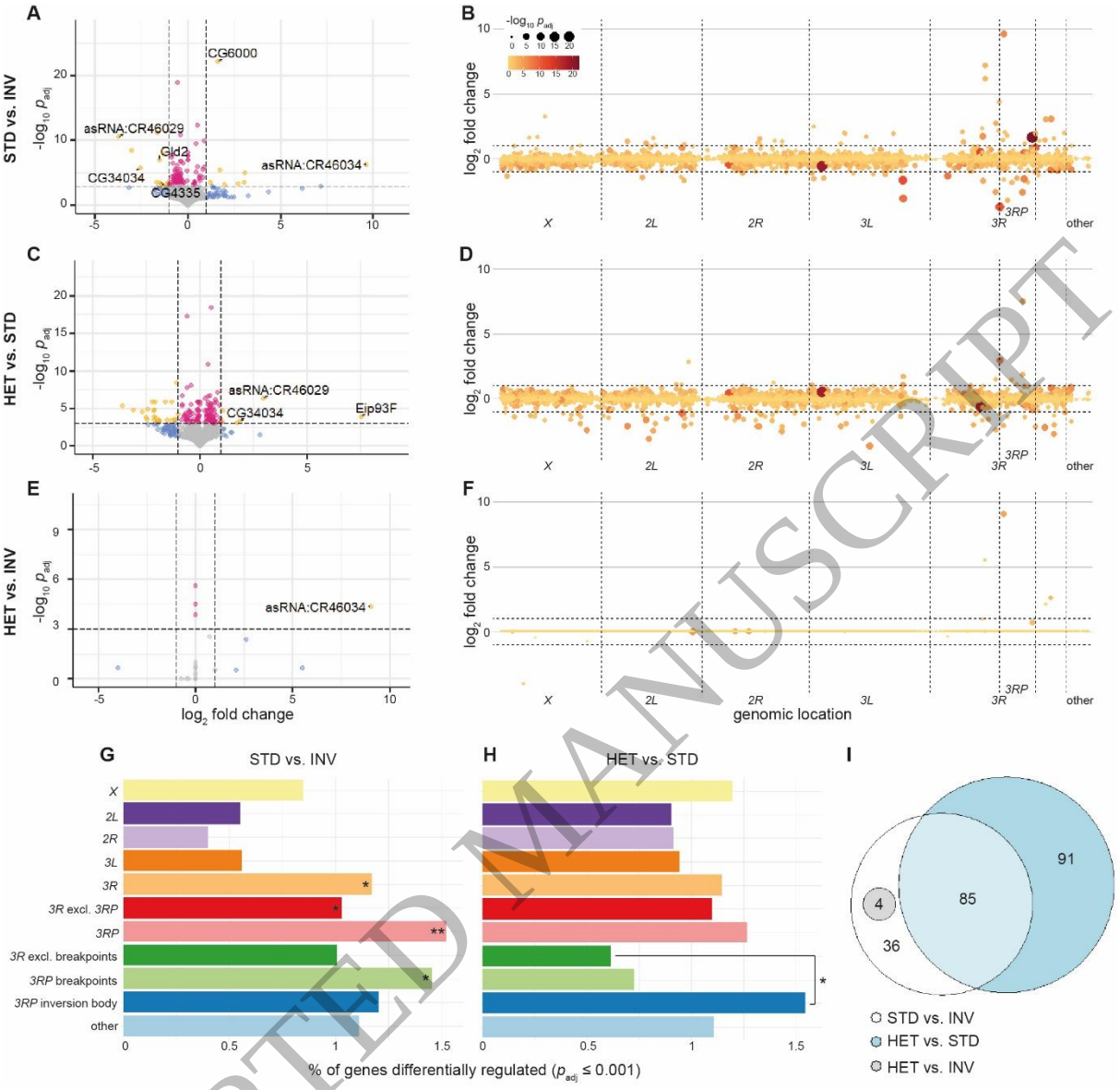

Figure 1
160x155 mm (x DPI)

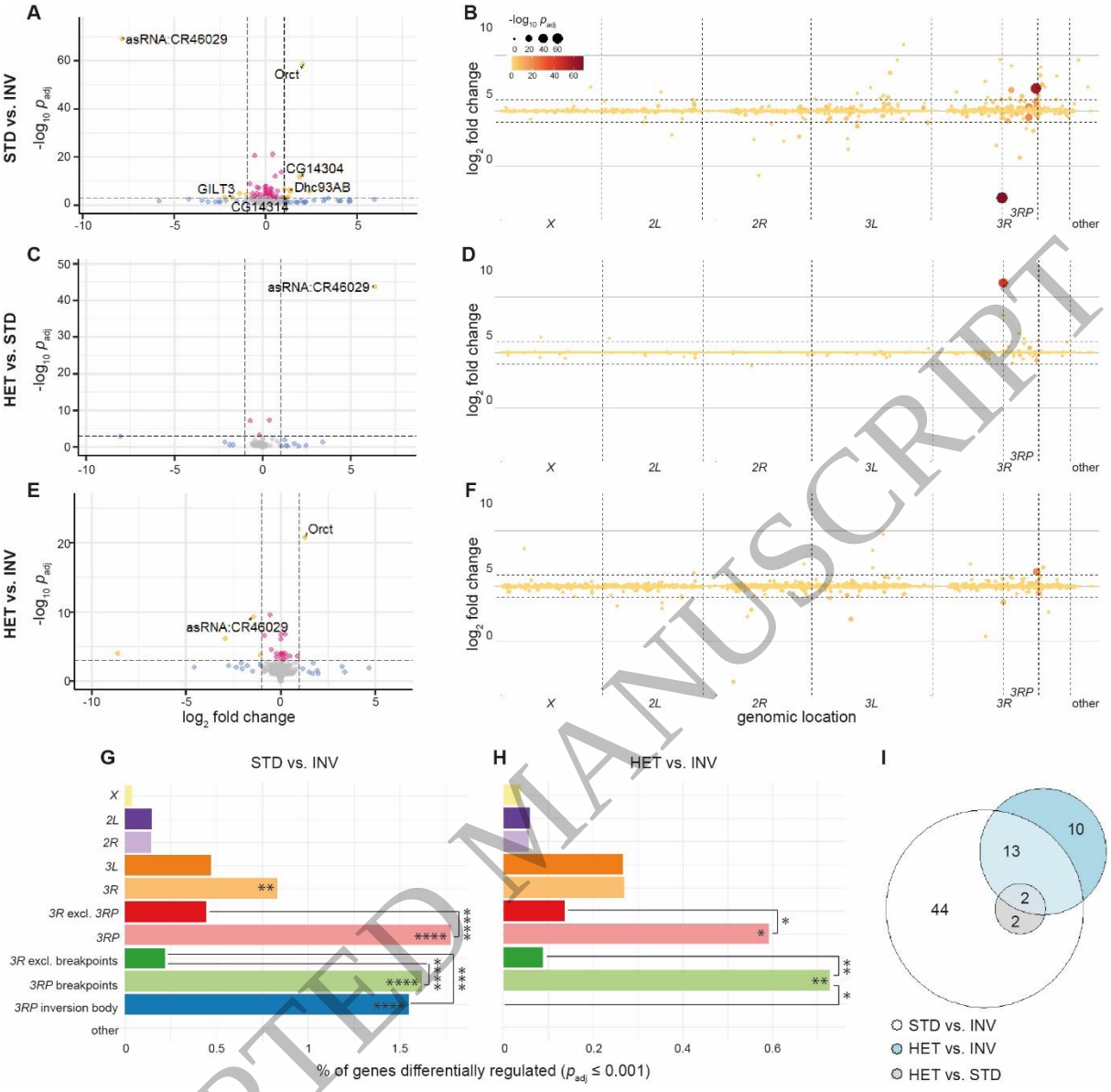

Figure 2
160x157 mm (x DPI)

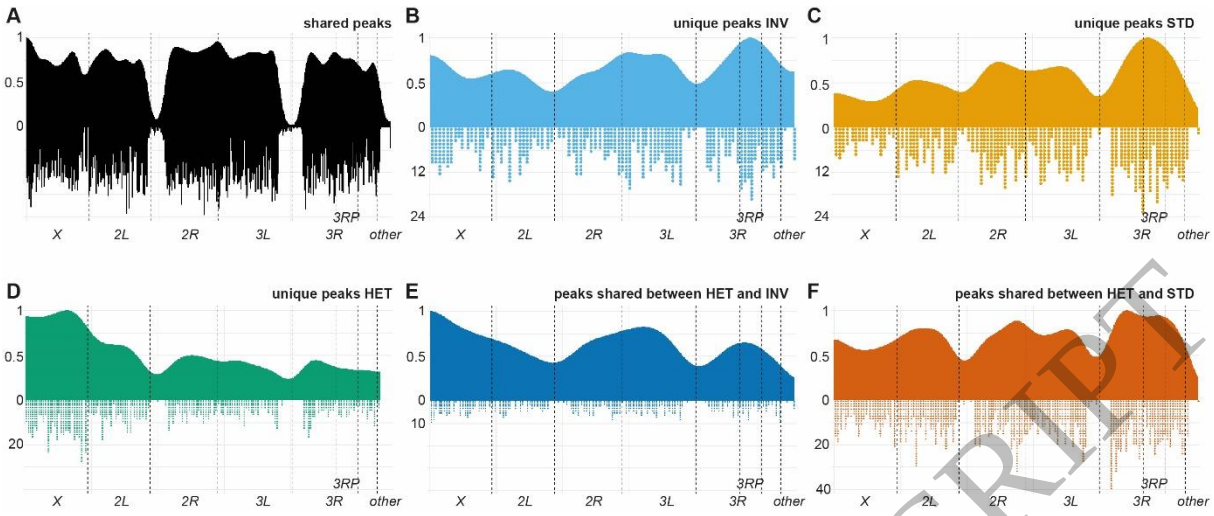

Figure 3
160x67 mm (x DPI)

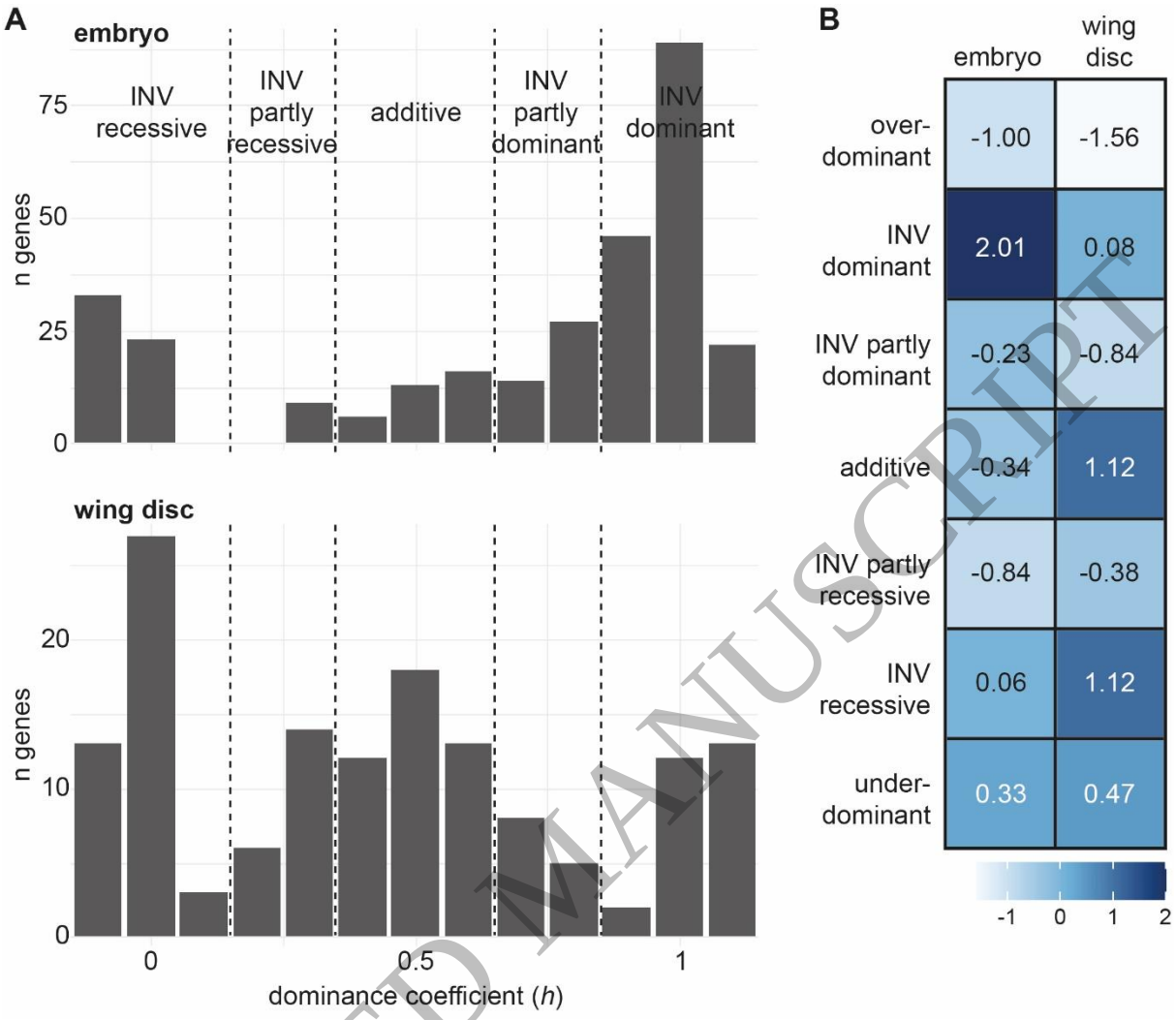

Figure 4
160x139 mm (x DPI)

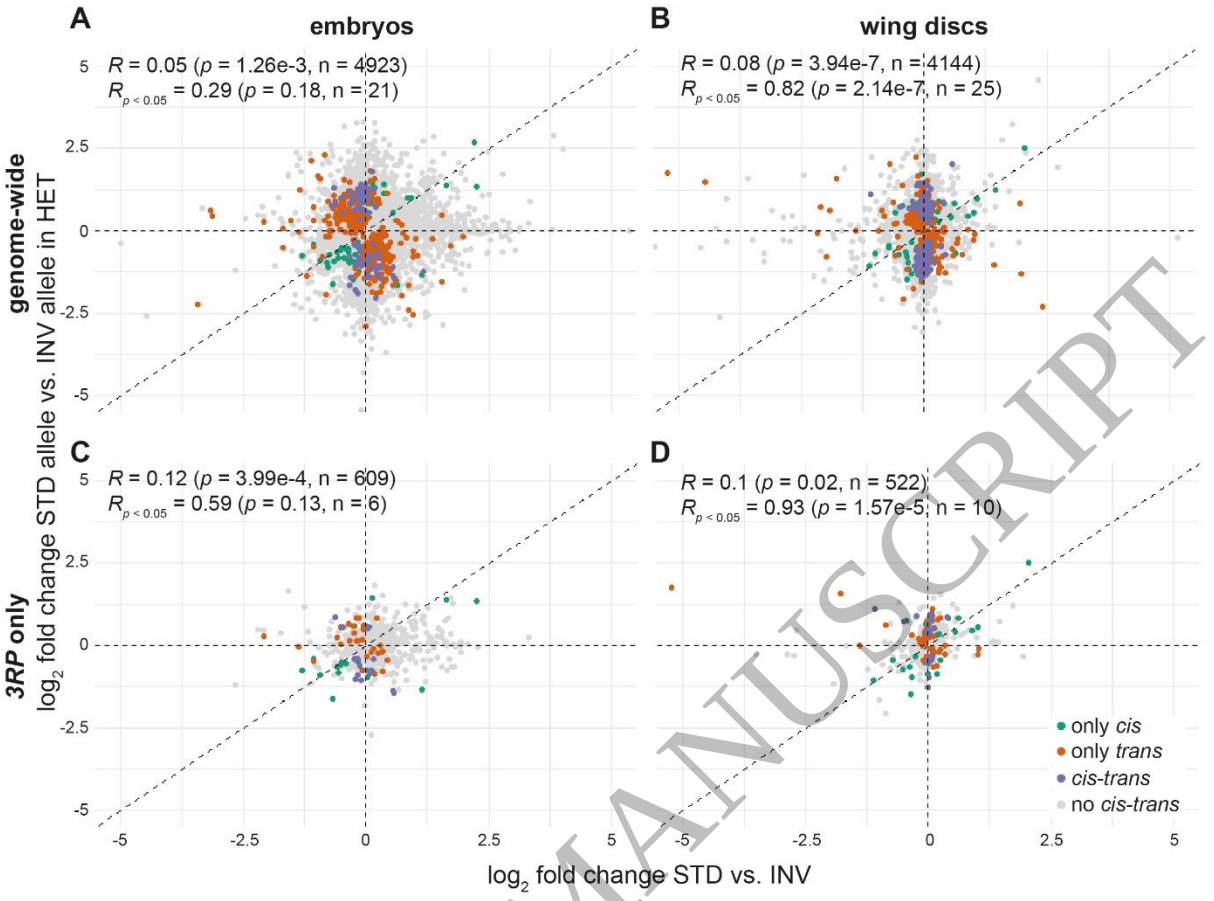

Figure 5
160x118 mm (x DPI)