RESEARCH

Meiotic drive against chromosome fusions in butterfly hybrids

Jesper Boman · Christer Wiklund · Roger Vila · Niclas Backström

Abstract Species frequently differ in the number and structure of chromosomes they harbor, but individuals that are heterozygous for chromosomal rearrangements may suffer from reduced fitness. Chromosomal rearrangements like fissions and fusions can hence serve as a mechanism for speciation between incipient lineages, but their evolution poses a paradox. How can rearrangements get fixed between populations if heterozygotes have reduced fitness? One solution is that this process predominantly occurs in

small and isolated populations, where genetic drift can override natural selection. However, fixation is also more likely if a novel rearrangement is favored by a transmission bias, such as meiotic drive. Here, we investigate chromosomal transmission distortion in hybrids between two wood white (*Leptidea sinapis*) butterfly populations with extensive karyotype differences. Using data from two different crossing experiments, we uncover that there is a transmission bias favoring the ancestral chromosomal state for derived fusions, a result that shows that chromosome fusions actually can fix in populations despite being counteracted by meiotic drive. This means that meiotic drive not only can promote runaway chromosome number evolution and speciation, but also that it can be a conservative force acting against karyotypic change and the evolution of reproductive isolation. Based on our results, we suggest a mechanistic model for why chromosome fusion mutations may be opposed by meiotic drive and discuss factors contributing to karyotype evolution in Lepidoptera.

Keywords Chromosomal rearrangements · Meiotic drive · Lepidoptera · Speciation · Karyotype · *Leptidea*

J. Boman (✉) · N. Backström
Evolutionary Biology Program, Department of Ecology and Genetics (IEG), Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden
e-mail: jesper.boman@ebc.uu.se

N. Backström
e-mail: niclas.backstrom@ebc.uu.se

C. Wiklund
Department of Zoology: Division of Ecology, Stockholm University, Stockholm, Sweden
e-mail: christer.wiklund@zoologi.su.se

R. Vila
Institut de Biologia Evolutiva (CSIC-Univ. Pompeu Fabra), Passeig Marítim de La Barceloneta 37-49, 08003 Barcelona, Spain
e-mail: roger.vila@csic.es

Introduction

Major chromosomal rearrangements leading to karyotypic differences (i.e. changes in chromosome number

or overall chromosome structure) can be important for the evolution of reproductive isolation and maintenance of species integrity. The underlying assumption to this argument is that individuals that are heterozygous for different chromosomal arrangements (heterokaryotypic individuals) should experience reduced fertility as a consequence of segregation problems during meiosis (see Fig. S1 for an example). While chromosomal rearrangements that lead to reduced fitness when in heterozygous state (a scenario generally referred to as underdominance) may constitute powerful barriers to gene flow between divergent lineages (King 1993; Deineri et al. 2003), the evolution of such karyotypic changes is paradoxical. How can chromosomal rearrangements reach fixation in a population when heterokaryotypic individuals have reduced fitness? Theoretical work has shown that fixation of such underdominant chromosomal rearrangements can occur in isolated populations with small effective population size (N_e) where allele frequency change predominantly is caused by genetic drift (Lande 1979; Walsh 1982; Gavrilets 2004). For this reason, the general importance of chromosome evolution in speciation processes has been questioned (Futuyma and Mayer 1980; Templeton 1981; Nei et al. 1983). However, the probability of fixation of an underdominant chromosomal rearrangement will increase if the rearranged chromosome structure is favored by a transmission bias, such as meiotic drive (White 1968). A novel rearrangement will predominantly occur in a heterozygous state. This is the critical phase for an underdominant rearrangement, since once it reaches an allele frequency of 0.5, it will experience the same average selection pressure as the ancestral arrangement. A transmission bias, such as meiotic drive, may favor either the novel or the ancestral variant in heterokaryotypes and consequently affect the fixation probability of different chromosomal rearrangements. Meiotic drive can therefore either oppose or mediate the evolution of chromosome number differences and reproductive isolation between species.

Previous studies suggest that meiotic drive could be a common evolutionary force (Smith 1976; Henikoff et al. 2001; Pardo-Manuel de Villena and Sapienza 2001; Burt and Trivers 2006; Kern et al. 2015; Wei et al. 2017; Stewart et al. 2019). An observation supporting this hypothesis comes from mammals, where most species tend to have either acrocentric (centromere located close to one end) or metacentric (centromere located close to the center) chromosomes (Pardo-Manuel de Villena and Sapienza 2001). If karyotype structure has evolved in a neutral fashion, we would rather expect a unimodal distribution, with a mix of of acrocentric/metacentric chromosomes. One opportunity for meiotic drive in females arises due to polar body formation, i.e. the production of primordial egg cells that never get fertilized. Chromosomes that are preferentially segregating to the mature egg cell rather than to the polar bodies will be transmitted to the offspring with a higher probability and can therefore increase in frequency in a population. In taxa with a single centromere per chromosome (monocentric), the spindle fibers attach to the kinetochore structure around the localized centromere during meiotic division and differences between homologous chromosomes in kinetochore size may therefore cause meiotic drive (Akera et al. 2017). In this case, chromosomal rearrangements may induce meiotic drive since fused and unfused chromosomes can differ in the amount of centromeric DNA and the recruitment of kinetochore proteins (Wu et al. 2018). While such "centromere drive" can result in karyotypic change, selfish centromeres seem to occur rather frequently and not only in fission/fusion heterokaryotypes (Henikoff et al. 2001; Dudka and Lampson 2022). This conclusion rests on the observation that both centromere sequences and the interacting kinetochore proteins have evolved rapidly in many taxa, while their function has been conserved (Henikoff et al. 2001). The molecular mechanism of centromere drive during female meiosis has been characterized in some detail in a few monocentric organisms (Chmátal et al. 2014; Akera et al. 2017, 2019; Clark and Akera 2021; Dudka and Lampson 2022). In contrast, little is known about the potential for meiotic drive and the underlying molecular mechanisms in organisms where centromere activity is distributed across numerous locations along chromosomes (holocentric) during meiosis (Bureš and Zedek 2014).

Butterflies and moths (Lepidoptera) have received a lot of attention in cytogenetic studies, partly due to the possibility of using the karyotype for species characterization (Lorković 1941; Lukhtanov and Dantchenko 2002; Lukhtanov et al. 2005; Descimon and Mallet 2009; Vila et al. 2010; Dincă et al. 2011). Lepidopterans have holocentric chromosomes in mitosis and female meiosis (Maeda 1939;

Suomalainen et al. 1973; Murakami and Imai 1974; Turner and Sheppard 1975; Rosin et al. 2021). Most lepidopteran species have a chromosome number close to $n=31$, but substantial variation exists (Lorković 1941; Lukhtanov 2014; de Vos et al. 2020). Macroevolutionary studies have shown that chromosome number variation is positively associated with the rate of speciation in some specific butterfly genera that have extensive karyotype differences between species (de Vos et al. 2020; Augustijnen et al. 2023). However, it is still unclear if the interspecific difference in karyotype is a result of genetic drift, natural selection, or some other fixation bias, such as meiotic drive. A few butterfly genera show especially extensive chromosome number variation. The wood white butterfly (*Leptidea sinapis*) has the greatest intraspecific variation in chromosome number of all diploid eukaryotes. *Leptidea sinapis* individuals in Catalonia (CAT) have $2n=106–108$, while Swedish (SWE) individuals of the same species have $2n=57, 58$ (Lukhtanov et al. 2011, 2018). Most of the interpopulation differences in karyotype spring from derived chromosome fissions and fusions in the CAT and SWE population, respectively (Höök et al. 2023) and there is a cline in chromosome number between these two extremes across Europe (Lukhtanov et al. 2011). In spite of the remarkable amount of rearrangements, hybrids between SWE and CAT are fertile and viable with hybrid breakdown of viability in F_2 and later generations indicative of recessive hybrid incompatibilities (Lukhtanov et al. 2018; Boman et al. 2023). These characteristics make *L. sinapis* an excellent model system for investigating the underlying evolutionary processes leading to karyotypic divergence. Hybrids are often used to investigate meiotic drive since drive systems are expected to rapidly lead to fixation or suppression by counter-adaptations (Hurst 2019; Fishman and Mcintosh 2019). In hybrids, dormant meiotic drivers may be released from suppression and drivers that have been fixed in the parental lineages may become observable due to reformation of heterozygosity (Phadnis and Orr 2009; Fishman and Mcintosh 2019). In addition, hybrids between SWE and CAT *L. sinapis* will be heterozygous for a large set of fissions and fusions. This can increase the overall power to detect transmission distortion, which may have a small effect on a per-generation timescale.

Here we performed crosses between SWE and CAT *L. sinapis* and sequenced a large set of F_2 offspring to assess potential transmission distortion (i.e. deviations from strict Mendelian segregation), to determine whether meiotic drive may be acting in this system. Our aims were to answer two main questions: i) Is there evidence for transmission distortion for chromosomes of a certain rearrangement type (e.g. fusion in the SWE lineage)? ii) Is potential transmission distortion favoring the ancestral or derived state at chromosomal rearrangements?

Materials and methods

Crossing experiments

We performed two crossing experiments between SWE and CAT *L. sinapis* (Fig. 1). First, pure lines of each population were crossed to form F_1 offspring. Two ♀SWE x ♂CAT and five ♀CAT x ♂SWE F_1 families were established by crossing offspring of wild-caught individuals from each parental line. Only males from the ♀SWE x ♂ CAT survived until the imago (adult) stage. The F_1 offspring were used to establish both an intercross (F_1 x F_1, $n=8$) and a backcross F_2-generation (F_1 female x male SWE, $n=2$; Table S1). Backcrossing was done to the SWE population out of convenience based on available individuals. For the intercross F_2 individuals, we monitored individual survival to determine the genomic architecture of hybrid inviability, following Boman et al. (2023). Here, all offspring ($n=599$) were sampled, i.e. both those that survived until adulthood and those that died at some stage during development. For the backcross families, we sampled all eggs that each female laid, three days after egg-laying ($n=32$ and $n=35$, per female).

DNA extraction and pooled sequencing

We extracted DNA from the F_2 hybrid offspring using a standard phenol–chloroform protocol. Some individuals that died during development and eggs were extracted in pools of 2–21 individuals, due to low total DNA content in e.g. dead embryos. We measured the DNA content of each extracted sample using Qubit, and pooled samples to get equimolar concentrations of each respective individual. For the intercross, five different pools of F_2 individuals

Fig. 1 Overview of the experimental crosses and rearrangement types. **A** Crossing design and expected allele frequencies in the presence or absence of transmission distortion. Ovals represent an example of a homologous pair of autosomes. Note that female meiosis in butterflies is achiasmatic, i.e. recombination occurs only in males. Consequently, the F_2 backcross is a test for female-specific transmission distortion. **B** Schematics of different rearrangement types
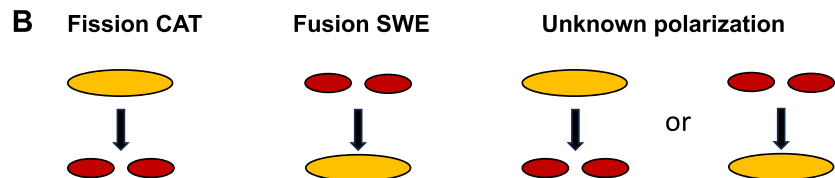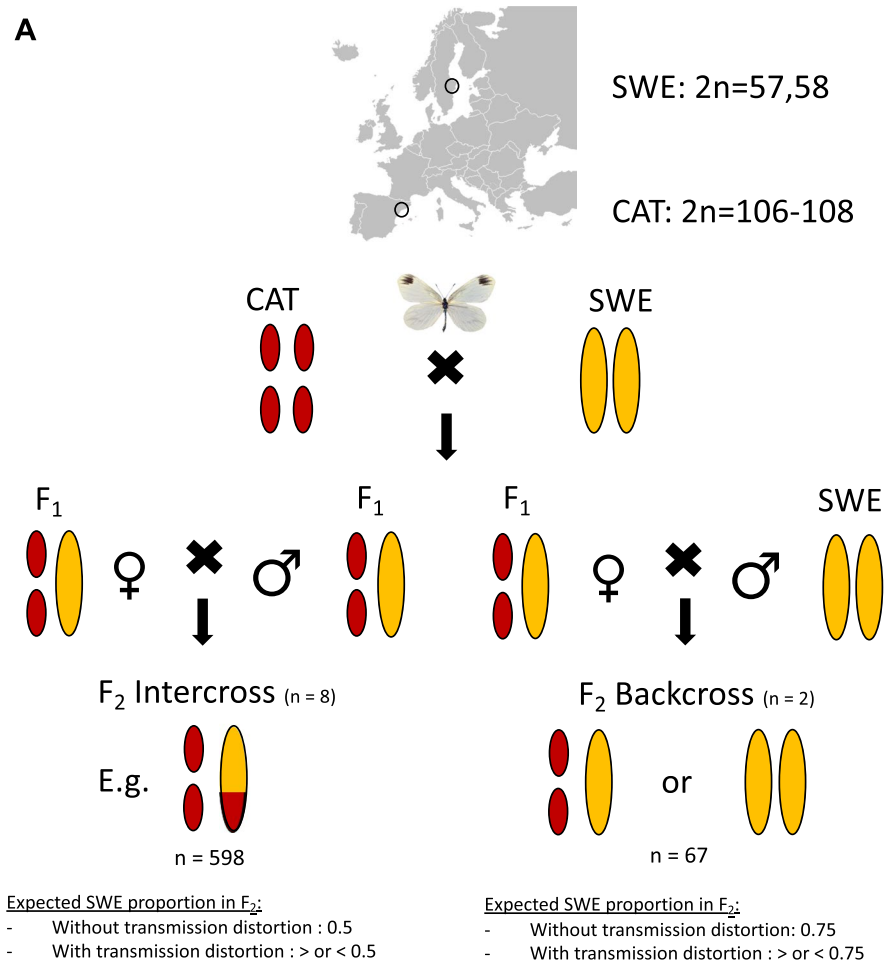
were sequenced: dead embryos ($n = 298$), eggs ($n = 73$), dead larvae + dead pupae ($n = 72$), adult males ($n = 76$) and adult females ($n = 80$). The egg pool for the F_2 intercross as well as eggs from the F_2 backcross were sampled three days after laying. Pools were prepared for sequencing using the Illumina TruSeq PCR-free library preparation method and whole-genome re-sequenced (2×151 bp paired-end reads with 350 bp insert size) on a single Illumina NovaSeq6000 (S4 flowcell) lane at NGI, SciLifeLab, Stockholm (Table S2).

Inference of fixed differences

To measure transmission distortion in the offspring we used genetic markers and estimated allele frequency differences compared to the expected value based on each type of cross. This means that in the F_2 backcross experiment we only tested transmission distortion in the F_1 hybrid female meiosis, while both male and female meiosis was tested in the F_2 intercross (Fig. 1). We inferred fixed differences between the parental populations using population re-sequencing data from 10 SWE and 10 CAT male

L. sinapis (Talla et al. 2019). In-depth information on variant calling can be found in Boman et al. (2023). Briefly, reads were trimmed and filtered and mapped to the Darwin Tree of Life reference genome assembly of a male *L. sinapis* from Asturias in north-west Spain, which is inferred to have a diploid chromosome number of 96 (Lohse et al. 2022). In total, we inferred 27,720 fixed differences distributed across all chromosomes.

Pool-seq read mapping and variant calling

We trimmed pool-seq reads and removed adapters using TrimGalore ver. 0.6.1, a wrapper for Cutadapt ver. 3.1 (Martin 2011). Seven base pairs (bp) were removed from the 3' end of each read and all reads with an overall Phred score < 30 were discarded. Filtered reads were mapped to two modified versions of the reference genome assembly, where all fixed differences were set to either the SWE allele or the CAT allele, respectively. For subsequent analysis, we used the average allele frequency of both mappings to mitigate the effects of potential assembly biases. For the mapping, we used bwa *mem* ver. 0.7.17 (Li 2013). Mapped reads were deduplicated using Picard *MarkDuplicates* ver. 2.23.4 and reads with a mapping quality < 20 were discarded (Schlötterer et al. 2014). Variant calling was performed with MAPGD ver. 0.5 *pool* and only variants with a likelihood ratio score < 10^{-6} were retained (Lynch et al. 2014). In the presentation of the results, we arbitrarily decided to show the allele frequencies of the SWE allele for each respective marker in the pools of sequenced individuals. The number of loci that were retained for analysis after filtering were 27,713 in the backcross and 27,533 in the intercross experiment, respectively.

Inference of transmission distortion

Rearrangement type classification was determined using parsimony based on synteny analyses between genome assemblies of *L. sinapis* and the related congenerics *L. reali* and *L. juvernica* (Höök et al. 2023; Näsvall et al. 2023). We inferred the degree of transmission distortion for four classes of rearrangements: derived fissions in the CAT population (Fission CAT), derived fusions in the SWE population (Fusion SWE), chromosomes with the two states segregating in all three *Leptidea* species (unknown polarization)

and homologous autosomes (Fig. 1B). Note that SWE has the fused and CAT has the unfused state for all chromosomes with unknown polarization. We used these groups to increase the power for detecting small effect transmission distortions (see Table S3 for a list of sample sizes per group). Note that the *L. sinapis* karyotype includes three Z-chromosomes (Šíchová et al. 2015) and those were excluded since they are monomorphic for the SWE state in the backcross. To accommodate for the undefined order of events in complex rearrangements we restricted our analysis to chromosome units with a 1:2 ratio, i.e. where chromosome states in the two populations differ by a single fission/fusion event. Transmission distortion was evaluated using two-tailed binomial tests in *R* ver. 4.2.2 (R Core Team 2020). To produce counts of chromosomes from observed allele frequencies we rounded allele frequencies per pair for chromosomes with a fission/fusion rearrangement. Thus, for the sample size in the binomial tests, we counted pairs, since we conservatively assumed that the underlying mechanism (such as holokinetic drive) affects both unfused chromosomes equally and consequently there is only one event per homologous bivalent or trivalent during meiosis.

Inference of ploidy

Patterns of transmission distortion can be caused by many processes, among them aneuploidy. We used pool-seq read counts at fixed differences to scan for the possibility of aneuploidy. If aneuploidy causes transmission distortion for a specific category of chromosomes, a higher sequencing read coverage for that category compared to other chromosome categories is expected. We therefore tested for significant differences in read coverage using both ANOVA and post-hoc analyses in *R*.

Results

Transmission distortion of derived fusions

We assessed potential transmission distortion in the F$_2$ offspring from crosses between SWE and CAT *L. sinapis* using a pool-seq approach (Table S2). The average allele frequencies in the F$_2$ offspring for all marker loci (fixed alleles between the parental

populations) were used to estimate potential deviations from strict Mendelian segregation using binomial tests. The analysis revealed significant transmission distortions for chromosomes with a derived fusion in the SWE lineage in both the F_2 backcross ($p \approx 0.028$) and the F_2 intercross ($p \approx 0.024$) (Table 1, Fig. 1 and Table S4). In both cases, the unfused chromosome state characteristic for the CAT population was significantly overrepresented. This pattern was not driven by any specific outlier chromosome(s), since all except one chromosome (SWE) or chromosome pair (CAT) showed consistent deviations towards the CAT chromosome state (Fig. 2). In the

intercross, we also observed a significant transmission distortion for chromosomes with unknown polarization in the direction of the fused SWE state ($p \approx 0.003$). Next, we considered explanations for the observed distortions. Since only Fusion SWE showed a significant deviation towards the CAT allele, it is not likely that the pattern is caused by reference bias. To test if aneuploidy could explain the observed transmission distortion, we calculated the coverage at marker loci for all chromosomes in the reference assembly (Fig. S2). No significant differences between chromosome classes were observed, except between the Z chromosomes and the autosomes

Table 1 Expected and observed allele frequencies in the F_2 backcross and intercross experiments and the results from binomial tests

Experiment	Chromosome type	Expected frequency	Observed frequency	Lower 95% CI	Upper 95% CI	p value
Backcross	Fission CAT	0.75	0.761	0.712	0.806	0.659
Backcross	Fusion SWE	0.75	0.701	0.654	0.746	**0.028**
Backcross	Homologous	0.75	0.725	0.674	0.772	0.725
Backcross	Unknown polarization	0.75	0.731	0.674	0.783	0.481
Intercross	Fission CAT	0.5	0.497	0.479	0.516	0.798
Intercross	Fusion SWE	0.5	0.481	0.465	0.498	**0.024**
Intercross	Homologous	0.5	0.494	0.476	0.512	0.511
Intercross	Unknown polarization	0.5	0.531	0.511	0.551	**0.003**

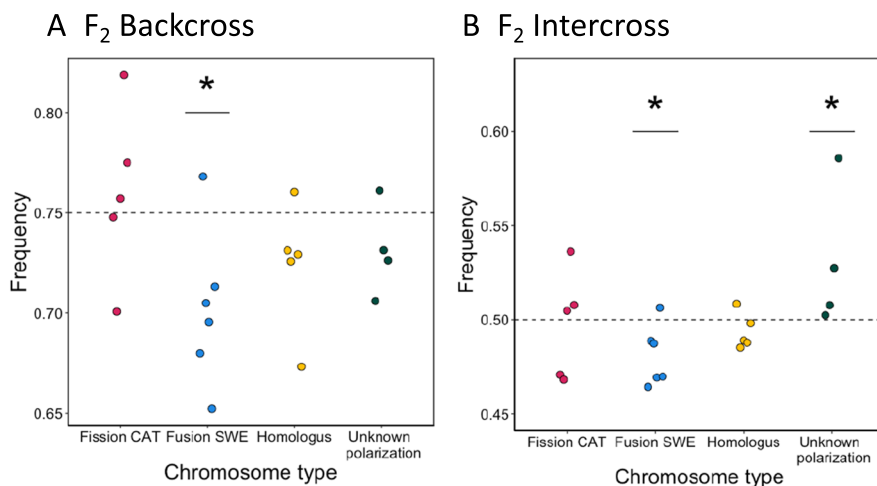Significant results are highlighted in bold

Fig. 2 Average allele frequencies at marker loci for each chromosome (or pair of chromosomes for fission/fusion heterozygotes) in the F_2 backcross (**A**) and the F_2 intercross (**B**). In all cases, SWE has the fused state and CAT has the unfused state, except for the homologous (not rearranged) chromosomes, where both populations have the same state. Dashed lines represent the expected allele frequency in each experiment. Points have dodged positions along the x-axis to enhance visibility. Rearrangement types with significant transmission distortion are marked with an asterisk (*)

(Table S5), which is expected since the W chromosome is highly degenerated in Lepidoptera. This indicates that systematic aneuploidy is not causing the observed transmission distortion in our data.

Discussion

Transmission distortion at derived fusions may be caused by female meiotic drive

Here we characterized transmission distortion using pool-seq of F_2 offspring from crosses between SWE and CAT *L. sinapis*. We observed transmission bias in both crossing experiments at derived fusions, supporting the significance of the results. The fact that we observed a bias in the F_2 backcross experiment suggest that female meiotic drive is causing the pattern at derived fusions. Mechanistically, the drive can be caused by differences in holokinetic binding of spindle fibers between the fused and unfused chromosome states, i.e. that the unfused ancestral state represented in the CAT population has stronger holokinetic activity. We only have rudimentary information available of the molecular components of the kinetochore structures and activities in Lepidoptera (Cortes-Silva

et al. 2020; Senaratne et al. 2021). Like other holocentric insects, it seems that butterflies and moths lack the centromeric histone H3 variant (CenH3, also known as CENP-A), which is otherwise ubiquitous among eukaryotes (Drinnenberg et al. 2014). In mitotic cell lines from the silk moth, *Bombyx mori*, the kinetochore formation is directed towards heterochromatic regions of the chromosomes (Senaratne et al. 2021). If kinetochore activity is similarly associated with heterochromatic regions during female meiosis in F_1 *L. sinapis* hybrids, it is possible that some unfused chromosomes have stronger centromeres due to proportionally more heterochromatin (Iwata-Otsubo et al. 2017). Chromosome fusion events might lead to loss of repetitive telomeric sequences at the fusion point (Fig. 3A). In line with this, it has been shown that telomere-associated LINE transposons only constitute 5% of all LINEs close to fusion points in both *L. sinapis* and the congeneric *L. reali*, indicating that DNA has been lost in those regions (Höök et al. 2023). It should be noted that the genome assemblies used for that repeat analysis were based on 10X linked-read sequences and not long-reads. Since the assemblers using 10X linked-reads often fail to scaffold repeat-rich sequences (Peona et al. 2021), the amount of repetitive (and putatively heterochromatic)
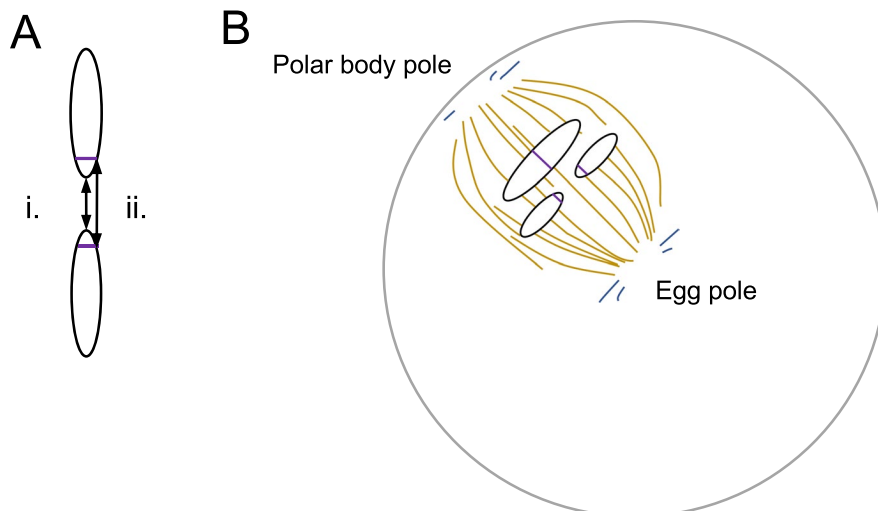

Fig. 3 A model that describes how meiotic drive can occur during female achiasmatic meiosis of holokinetic organisms. **A** A fusion could either form through joining of ends (i) or e.g. non-homologous recombination, leading to loss of heterochromatic sequence at the fusion point (ii). **B** The loss of heterochromatic sequence could lead to a weaker holocentromere, which results in biased segregation during meiosis, either towards the polar body pole or the egg pole. If this mechanism explains the observed transmission distortion, the probability that the stronger holocentromere (in this case the unfused chromosomes) ends up in the mature oocyte is higher

DNA at fusion breakpoints in *Leptidea* could be underestimated. If the meiotic drive observed for fused/unfused chromosome pairs is caused by differential kinetochore assembly due to loss of heterochromatin during fusion events, this can also explain why we did not detect any signal of meiotic drive for derived fissions. Fissions can form by double-strand breaks and are potentially not associated with the same heterochromatin differential between fused and unfused states. To conclusively test the hypothesis of holokinetic drive in *L. sinapis*, the next step will be to identify the kinetochore components and estimate the relative abundance of kinetochore proteins in meiotic cells in F_1 hybrid females (Chmátal et al. 2014). Ideally, the kinetochore content can then be manipulated to experimentally validate if differential assembly of the kinetochore causes drive or not.

An alternative explanation to the observed transmission distortion would be early acting embryo viability selection enriched at chromosome fusions. While it is possible, we find it less likely since that would require that loci underlying viability are selected in both the F_2 backcross and F_2 intercross experiments, despite the different genomic backgrounds in individuals from those crosses. While we cannot rule out such a scenario, we consider female meiotic drive to be a more parsimonious explanation for the biased allele frequency distributions observed at derived fusions here. We also observed a transmission distortion favoring the fused state (SWE) for chromosomes with unknown polarization, i.e. rearrangement polymorphisms that are segregating within both *L. sinapis* and the closely related species *L. reali* and *L. juvernica.* This pattern is probably not caused by female meiotic drive since we did not observe such a transmission bias in the F_2 backcross. This specific transmission distortion could potentially be caused by fertility selection on F_1 parents which likely is stronger in F_1 male than female meiosis in this system (Lukhtanov et al. 2018), but could also be a consequence of early embryo viability selection or meiotic drive in males.

Causes and consequences of karyotype evolution in Lepidoptera

The potential for meiotic drive to cause karyotype evolution has been appreciated in both monocentric (Pardo-Manuel de Villena and Sapienza 2001)

and holocentric organisms (Bureš and Zedek 2014). Here, we used a data set of almost 2,500 lepidopteran taxa (de Vos et al. 2020), to interpret our experimental evidence for transmission distortion for fission/fusion polymorphisms in *L. sinapis* (Fig. 4). A visual inspection shows that a haploid count (n) of 31 chromosomes is the most common karyotype in Lepidoptera, but also that there is a substantial variation in chromosome numbers. Genera with species having a comparatively high number of chromosomes tend to have a higher variance in chromosome numbers (Fig. 4, group i and ii). Only species within a few genera (*Leptidea* and *Polyommatus* sensu lato) have many members with high chromosome numbers (group i). A minority of species in group ii have $n > 31$ and a majority of genera comprise species with a maximum $n <= 31$ (group iii and iv). While no comprehensive phylogeny for the taxa included in this data set has been inferred, we can still use the information about chromosome number variation in Lepidoptera to draw a few conclusions. First, chromosome fusions are apparently widespread across Lepidoptera. This was recently confirmed by whole-genome alignments of more than 200 butterfly and moth species (Wright et al. 2024). Recent models of chromosomal speciation and the role of chromosomal rearrangements in local adaptation have shown that a reduced recombination rate caused by a fusion event could be favored by selection and lead to speciation (Navarro and Barton 2003; Kirkpatrick and Barton 2006; Guerrero and Kirkpatrick 2014). Consequently, while meiotic drive could be involved it is not necessarily needed to explain the numerous chromosome fusions across the tree of Lepidoptera. Second, very few Lepidoptera species have high chromosome numbers as a consequence of multiple chromosome fissions. The difference between the rate of accumulation of fissions and fusions cannot be explained by the hybrid underdominance model, which is perhaps of less importance in Lepidoptera than in e.g. mammals according to current evidence (Castiglia 2014; Lukhtanov et al. 2018; Hora et al. 2019). This indicates that other processes are facilitating accumulation of fissions in some clades. In both *Leptidea* and *Polyommatus*, which are the primary examples of species with highly fragmented karyotypes, inverted meiosis (i.e. sister chromatid segregation in meiosis I) has been observed (Lukhtanov and Dantchenko 2017; Lukhtanov et al. 2018, 2020a). It has been argued that
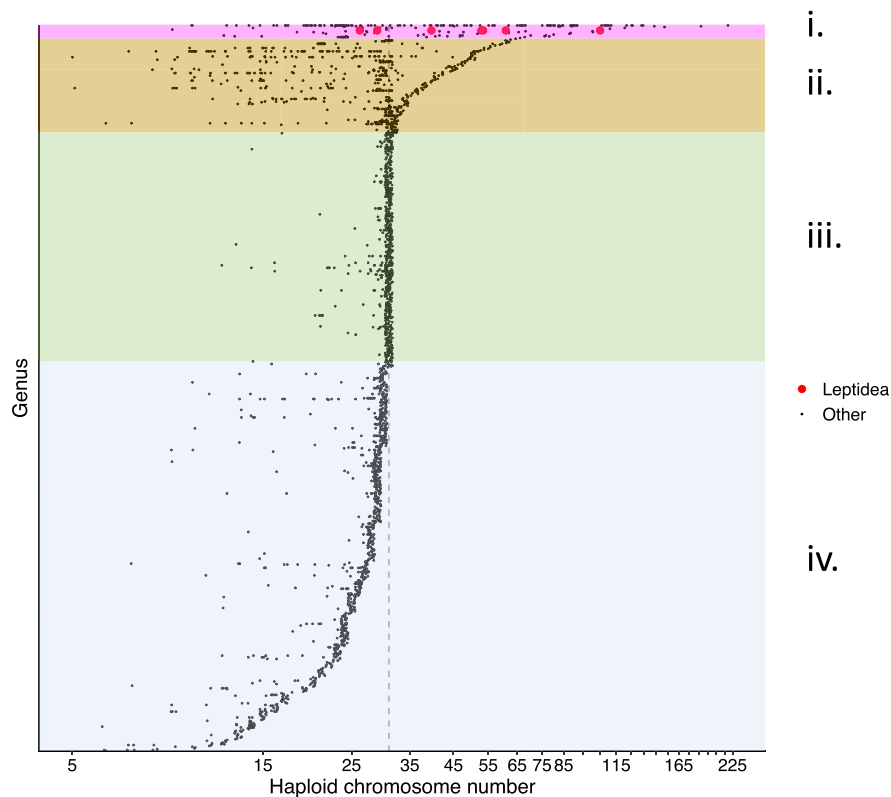
Fig. 4 Haploid chromosome number count of 2,499 lepidopteran taxa from 869 genera. The data is from de Vos et al. (2020) with information from two *Leptidea* species added (Lukhtanov et al. 2011). The dashed vertical line indicates $n = 31$, the most common karyotype within Lepidoptera. Genera are sorted by maximum chromosome number with points representing individual taxa. Groups i-iv represents rough categories of chromosome number distribution per genus. Group i consists of a few genera with great within-genus variation in chromosome number and many members with $n > 31$. Group ii genera have high max counts and great within-genus variation, but the distribution is generally skewed towards low numbers. Group iii genera show low within-genus variation, and most members have $n = 31$. Group iv genera have a max count < 31 with many genera having species with lower numbers. Points have slightly dodged position to enhance visualization of overlapping points. Haploid chromosome numbers are plotted on a log2 scale

while the achiasmatic (no crossover) female meiosis is sufficient to rescue fertility of trivalents, inverted meiosis could in some cases be necessary to rescue fertility (to some extent) in the chiasmatic male meiosis (Lukhtanov et al. 2018). Inverted meiosis in holocentric organisms can thus reduce the selective disadvantage of trivalents in meiosis, increasing the probability for fixation of both fissions and fusions (Table 2). However, we do not yet know if inverted meiosis is a widespread phenomenon in Lepidoptera and thus how general such fertility rescue processes might be, though some authors consider inverted meiosis an exception based on observations of meiotic karyotypes from hundreds of Lepidopteran species (Lukhtanov et al. 2020a). Inverted meiosis has also

been observed in some *Bombyx* moth karyotypes, but *Bombyx* does not show the same chromosome number variation as *Leptidea* and *Polyommatus* (Murakami and Imai 1974; Banno et al. 1995; de Vos et al. 2020). This demonstrates that inverted meiosis is not necessarily associated with rapid karyotype evolution. In *Leptidea sinapis,* chromosome number is positively associated with the genetic map length (Näsvall et al. 2023), i.e. populations with more chromosomes have a higher recombination rate per physical unit length. An increased recombination rate as a consequence of chromosome fragmentation can potentially be beneficial, since a higher recombination rate reduces the impact of selection on linked sites (Fisher 1930). Signatures of linked selection has been documented in

Table 2 Effects of different factors on karyotype evolution in Lepidoptera with special attention to the effects of meiotic drive

Factor	Effect	Consequence
Underdominant fissions/fusions	Imposes a fertility cost in hybrid meiosis	Decreased variability in chromosome number
Genetic drift	Random fixation of neutral fissions/fusions. In addition, novel underdominant fissions/fusions may fix if genetic drift overwhelms the negative fertility effect	Increased variability in chromosome number
Epistatic selection	Selection for the co-inheritance of combinations of alleles on different chromosomes	Decrease in chromosome number
Selective interference	Reduced efficacy of selection leading to selection for increased recombination	Increase in chromosome number
Holocentricity	Increased tolerance to chromosome fissions/fusions in female (achiasmatic) meiosis	Increased variability in chromosome number
Inverted meiosis	(Partially) rescued fitness of heterokaryotypes in male (chiasmatic) meiosis	Increased variability in chromosome number
Meiotic drive (If supporting derived arrangement)	Fixation bias during female meiosis	Increase or decrease in chromosome number
Meiotic drive (If supporting ancestral arrangement)	Fixation bias during female meiosis	Decreased variability in chromosome number
Meiotic errors	More chromosomes in meiosis leads to more opportunities for errors in meiosis	Decrease in chromosome number

L. sinapis (Boman et al. 2021; Näsvall et al. 2023). However, an increased recombination rate also leads to a higher probability that beneficial associations between alleles in linked regions are broken up. We speculate that a higher chromosome number may also increase the risk of mis-segregation during meiosis. Given the potential costs of increasing chromosome number, it is possible that maladaptive meiotic drive has played a role in biasing the fixation of unfused chromosomes.

Meiotic drive opposing fixation of derived fusions

Since we observed a bias for the fused state for chromosomes with unknown polarization and the unfused state for derived fusions, predicting what continued intercrossing would do to chromosome number in this system is difficult. A tendency towards a higher chromosome number has been observed in crosses between lepidopteran lineages with different karyotypes. In the closely related *Lysandra hispana* ($n=84$) and *L. coridon* ($n=88—90$), individuals tended to harbor the higher chromosome number after three generations of intercrossing (Beuret 1957). Similarly, in *Antheraea roylei* ($n=31$) and *A. pernyi* ($n=49$), intercrossed individuals in the F_{23} and F_{32} generations had $n=49$ (Nagaraju and Jolly 1986).

These results implicate that a fixation bias has been at play, since the expectation from genetic drift alone is the formation of a hybrid race with a karyotype distribution centered around the intermediate chromosome count (Lukhtanov et al. 2020b). In contrast to our study, the action of post-embryonic viability selection can however not be excluded in the crosses of *Lysandra* and *Antheraea*. In *L. sinapis*, we observed transmission distortion for derived fusions where the unfused chromosomes were overrepresented in the F_2 offspring. This result does not support previously suggested models where meiotic drive promotes karyotype evolution (Pardo-Manuel de Villena and Sapienza 2001; Bureš and Zedek 2014). Instead, our results support a model where derived fusions are opposed by meiotic drive, i.e. that meiotic drive can act as a conservative force. If this pattern can be extrapolated more widely across Lepidoptera it lends further credence to positive selection acting on chromosome fusions, since they would have to fix while opposed by meiotic drive (Mackintosh et al. 2023). However, we emphasize that meiotic drive may very well have promoted karyotype change in some lepidopteran lineages (such as *Antheraea*), but conclusive experimental evidence for this is lacking. Experimental analyses across a wider range of taxa are needed to draw definitive conclusions on the general role of

meiotic drive for karyotype evolution in Lepidoptera, but our results suggest that it may at least occasionally counteract karyotype change.

Meiotic drive may be opposing evolution of hybrid inviability

In a previous study, we mapped the genomic architecture of F_2 intercross hybrid inviability between the SWE and CAT chromosomal races of *L. sinapis* and observed a two-fold enrichment of candidate loci for hybrid inviability in derived fusion regions (Boman et al. 2023). This means that both transmission distortion and hybrid inviability are associated with the same chromosomes regions in this system, a pattern that has not been observed before as far as we know. However, genomic co-localization of regions affected by male meiotic drive and loci underlying hybrid sterility has been observed before in crosses between *Drosophila* taxa (Hauschteck-Jungen 1990; Tao et al. 2001; Phadnis and Orr 2009). It is believed that meiotic drive can promote the evolution of hybrid sterility through the formation of different driver-suppressor systems in divergent lineages experiencing limited gene flow (Frank 1991; Hurst and Pomiankowski 1991). Upon secondary contact, driver-suppressor systems could be misregulated and cause sterility in hybrids. While meiotic drive is intimately linked to reproductive processes, similar arguments could to some extent also be applied to hybrid inviability (Frank 1991; Hurst and Pomiankowski 1991). If meiotic drive accelerates sequence divergence, hybrid incompatibility could evolve as by-product of pleiotropy or physical linkage between the hybrid incompatibility locus and a driver or a suppressor. Conversely, since we observed meiotic drive in *L. sinapis* with a predisposition for the ancestral arrangement, it is possible that the factors contributing to hybrid inviability have evolved despite the counteracting force of meiotic drive. Consequently, the meiotic drive in the *L. sinapis* system could be opposing rather than promoting speciation. A similar pattern has previously been observed in *D. simulans* and *D. mauritiana*, where a driver has introgressed between species, which has resulted in reduced sequence divergence in that specific region (Meiklejohn et al. 2018). An alternative explanation would be that a substitution contributing to hybrid inviability reached high frequencies in the CAT population.

Indeed, substitutions at Fusion SWE chromosomes in both populations could be contributing to hybrid inviability. More detailed characterization of the genetic basis of hybrid inviability is needed to further clarify the relationship between reproductive isolation and meiotic drive in this system.

Declarations

Ethics approval   Not applicable.

Consent to participate   Not applicable.

Consent for publication   Not applicable.

Competing interests   The authors declare no competing interests.

References

Akera T, Chmátal L, Trimm E et al (2017) Spindle asymmetry drives non-Mendelian chromosome segregation. Science 358:668–672. https://doi.org/10.1126/science.aan0092

Akera T, Trimm E, Lampson MA (2019) Molecular strategies of meiotic cheating by selfish centromeres. Cell 178:1132-1144.e10. https://doi.org/10.1016/j.cell.2019.07.001

Augustijnen H, Bätscher L, Cesanek M et al (2023) A macroevolutionary role for chromosomal fusion and fission in Erebia butterflies. bioRxiv 2023.01.16.524200. https://doi.org/10.1101/2023.01.16.524200

Banno Y, Yutaka K, Koga K, Doira H (1995) Postreductional meiosis revealed in males of the mutant with chromosomal aberration "T (23;25) Nd" of the silkworm Bombyx mori. J Sericultural Sci Japan 64:410–414. https://doi.org/10.11416/kontyushigen1930.64.410

Beuret H (1957) Studien über den Formenkreis Lysandra coridon-hispana-albicans. Ein Beitrag zum Problem der Artbildung (Lep. Lycaenidae) (2. Studie). Mitteilungen der Entomol. Gesellschaft Basel 7 N.F:37–60

Boman J, Mugal CF, Backström N (2021) The effects of GC-biased gene conversion on patterns of genetic diversity among and across butterfly genomes. Genome Biol Evol 13. https://doi.org/10.1093/gbe/evab064

Boman J, Näsvall K, Vila R et al (2023) Evolution of hybrid inviability associated with chromosome fusions. bioRxiv 2023.11.30.569355. https://doi.org/10.1101/2023.11.30.569355

Bureš P, Zedek F (2014) Holokinetic drive: Centromere drive in chromosomes without centromeres. Evolution 68:2412–2420. https://doi.org/10.1111/evo.12437

Burt A, Trivers R (2006) Genes in conflict: the biology of selfish genetic elements. Belknap Press: An Imprint of Harvard University Press, Cambridge, Massachusetts

Castiglia R (2014) Sympatric sister species in rodents are more chromosomally differentiated than allopatric ones: Implications for the role of chromosomal rearrangements in speciation. Mamm Rev 44:1–4. https://doi.org/10.1111/MAM.12009/SUPPINFO

Chmátal L, Gabriel SI, Mitsainas GP et al (2014) Centromere strength provides the cell biological basis for meiotic drive and karyotype evolution in mice. Curr Biol 24:2295–2300. https://doi.org/10.1016/j.cub.2014.08.017

Clark FE, Akera T (2021) Unravelling the mystery of female meiotic drive: where we are. Open Biol 11. https://doi.org/10.1098/RSOB.210074

Cortes-Silva N, Ulmer J, Kiuchi T et al (2020) CenH3-independent kinetochore assembly in Lepidoptera requires CCAN, including CENP-T. Curr Biol 30:561-572.e10. https://doi.org/10.1016/j.cub.2019.12.014

de Vos JM, Augustijnen H, Bätscher L, Lucek K (2020) Speciation through chromosomal fusion and fission in Lepidoptera. Philos Trans R Soc B Biol Sci 375:20190539. https://doi.org/10.1098/rstb.2019.0539

Deineri D, Colson I, Grammenoudi S et al (2003) Engineering evolution to study speciation in yeasts. Nature 422:68–72. https://doi.org/10.1038/nature01418

Descimon H, Mallet J (2009) Bad species. Ecol Butterflies Eur 500:219

Dincă V, Lukhtanov VA, Talavera G, Vila R (2011) Unexpected layers of cryptic diversity in wood white Leptidea butterflies. Nat Commun 2:324. https://doi.org/10.1038/ncomms1329

Drinnenberg IA, DeYoung D, Henikoff S, Malik HS ing (2014) Recurrent loss of CenH3 is associated with independent transitions to holocentricity in insects. Elife 3. https://doi.org/10.7554/ELIFE.03676

Dudka D, Lampson MA (2022) Centromere drive: model systems and experimental progress. Chromosom Res 30:187–203. https://doi.org/10.1007/S10577-022-09696-3/FIGURES/6

Fisher RA (1930) The genetical theory of natural selection. Clarendon Press, Oxford

Fishman L, Mcintosh M (2019) Standard deviations: the biological bases of transmission ratio distortion. Annu Rev Genet 53:347–372

Frank SA (1991) Divergence of meiotic drive-suppression systems as an explanation for sex-biased hybrid sterility and inviability. Evolution 45:262–267. https://doi.org/10.1111/j.1558-5646.1991.tb04401.x

Futuyma DJ, Mayer GC (1980) Non-allopatric speciation in animals. Syst Zool 29:254. https://doi.org/10.2307/2412661

Gavrilets S (2004) Fitness landscapes and the origin of species. Princeton University Press, Princeton, New Jersey

Guerrero RF, Kirkpatrick M (2014) Local adaptation and the evolution of chromosome fusions. Evolution 68:2747–2756. https://doi.org/10.1111/evo.12481

Hauschteck-Jungen E (1990) Postmating reproductive isolation and modification of the "sex ratio" trait in Drosophila subobscura induced by the sex chromosome gene arrangement A2+3+5+7. Genetica 83:31–44. https://doi.org/10.1007/BF00774686/METRICS

Henikoff S, Ahmad K, Malik HS et al (2001) The centromere paradox: stable inheritance with rapidly evolving DNA. Science 293:1098–1102. https://doi.org/10.1126/science.1062939

Höök L, Näsvall K, Vila R et al (2023) High-density linkage maps and chromosome level genome assemblies unveil direction and frequency of extensive structural rearrangements in wood white butterflies (Leptidea spp.). Chromosom Res 31:1–23. https://doi.org/10.1007/S10577-023-09713-Z

Hora KH, Marec F, Roessingh P, Menken SBJ (2019) Limited intrinsic postzygotic reproductive isolation despite chromosomal rearrangements between closely related sympatric species of small ermine moths (Lepidoptera:

Yponomeutidae). Biol J Linn Soc 128:44–58. https://doi.org/10.1093/BIOLINNEAN/BLZ090

Hurst LD (2019) A century of bias in genetics and evolution. Heredity (edinb) 123:33–43. https://doi.org/10.1038/s41437-019-0194-2

Hurst LD, Pomiankowski A (1991) Causes of sex ratio bias may account for unisexual sterility in hybrids: A new explanation of Haldane's rule and related phenomena. Genetics 128:841–858. https://doi.org/10.1093/genetics/128.4.841

Iwata-Otsubo A, Dawicki-McKenna JM, Akera T et al (2017) Expanded satellite repeats amplify a discrete CENP-A nucleosome assembly site on chromosomes that drive in female meiosis. Curr Biol 27:2365-2373.e8. https://doi.org/10.1016/J.CUB.2017.06.069

Kern P, Cook JM, Kageyama D, Riegler M (2015) Double trouble: combined action of meiotic drive and Wolbachia feminization in Eurema butterflies. Biol Lett 11. https://doi.org/10.1098/rsbl.2015.0095

King M (1993) Species evolution: role of chromosome change. Cambridge University Press, Cambridge

Kirkpatrick M, Barton N (2006) Chromosome inversions, local adaptation and speciation. Genetics 173:419–434. https://doi.org/10.1534/GENETICS.105.047985

Lande R (1979) Effective deme sizes during long-term evolution estimated from rates of chromosomal rearrangement. Evolution 33:234. https://doi.org/10.2307/2407380

Li H (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 1303. https://doi.org/10.48550/arxiv.1303.3997

Lohse K, Höök L, Näsvall K, Backström N (2022) The genome sequence of the wood white butterfly, Leptidea sinapis (Linnaeus, 1758). Wellcome Open Res 7:254. https://doi.org/10.12688/WELLCOMEOPENRES.18118.1

Lorković Z (1941) Die Chromosomenzahlen in der Spermatogenese der Tagfalter. Zeitschrift Für Zellforsch Und Mikroskopische Anat Abt B Chromosom 21(2):155–191. https://doi.org/10.1007/BF00325958

Lukhtanov VA (2014) Chromosome number evolution in skippers (Lepidoptera, Hesperiidae). Comp Cytogenet 8:275–291. https://doi.org/10.3897/CompCytogen.v8i4.8789

Lukhtanov VA, Dantchenko AV (2002) Principles of the highly ordered arrangement of metaphase I bivalents in spermatocytes of Agrodiaetus (Insecta, Lepidoptera). Chromosom Res 10:5–20. https://doi.org/10.1023/A:1014249607796/METRICS

Lukhtanov VA, Kandul NP, Plotkin JB et al (2005) Reinforcement of pre-zygotic isolation and karyotype evolution in Agrodiaetus butterflies. Nature 436:385–389. https://doi.org/10.1038/nature03704

Lukhtanov VA, Dincă V, Talavera G, Vila R (2011) Unprecedented within-species chromosome number cline in the Wood White butterfly Leptidea sinapis and its significance for karyotype evolution and speciation. BMC Evol Biol 11:109. https://doi.org/10.1186/1471-2148-11-109

Lukhtanov VA, Dinca V, Friberg M et al (2018) Versatility of multivalent orientation, inverted meiosis, and rescued fitness in holocentric chromosomal hybrids. Proc Natl Acad Sci U S A 115:E9610–E9619. https://doi.org/10.1073/pnas.1802610115

Lukhtanov VA, Dantchenko AV, Khakimov FR et al (2020a) Karyotype evolution and flexible (conventional versus inverted) meiosis in insects with holocentric chromosomes: a case study based on Polyommatus butterflies. Biol J Linn Soc 130:683–699. https://doi.org/10.1093/BIOLINNEAN/BLAA077

Lukhtanov VA, Dincă V, Friberg M et al (2020b) Incomplete sterility of chromosomal hybrids: implications for karyotype evolution and homoploid hybrid speciation. Front Genet 11:1205. https://doi.org/10.3389/fgene.2020.583827

Lukhtanov VA, Dantchenko AV (2017) A new butterfly species from south Russia revealed through chromosomal and molecular analysis of the Polyommatus (Agrodiaetus) damonides complex (Lepidoptera, Lycaenidae). Comp Cytogenet 11(4) 769–795. https://doi.org/10.3897/COMPCYTOGEN.V11I4.20072

Lynch M, Bost D, Wilson S et al (2014) Population-Genetic Inference from Pooled-Sequencing Data. Genome Biol Evol 6:1210–1218. https://doi.org/10.1093/GBE/EVU085

Mackintosh A, Vila R, Martin SH et al (2023) Do chromosome rearrangements fix by genetic drift or natural selection? Insights from Brenthis butterflies. Mol Ecol. https://doi.org/10.1111/MEC.17146

Maeda T (1939) Chiasma Studies in the Silkworm, Bombyx mori L. Japanese J Genet 15:118–127. https://doi.org/10.1266/jjg.15.118

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10. https://doi.org/10.14806/ej.17.1.200

Meiklejohn CD, Landeen EL, Gordon KE et al (2018) Gene flow mediates the role of sex chromosome meiotic drive during complex speciation. Elife 7. https://doi.org/10.7554/eLife.35468

Murakami A, Imai HT (1974) Cytological evidence for holocentric chromosomes of the silkworms, Bombyx mori and B. mandarina, (Bombycidae, Lepidoptera). Chromosoma 47:167–178. https://doi.org/10.1007/BF00331804

Nagaraju J, Jolly MS (1986) Interspecific hybrids of Antheraea roylei and A. pernyi - a cytogenetic reassessment. Theor Appl Genet 72:269–273. https://doi.org/10.1007/BF00267003/METRICS

Näsvall K, Boman J, Höök L et al (2023) Nascent evolution of recombination rate differences as a consequence of chromosomal rearrangements. PLOS Genet 19:e1010717. https://doi.org/10.1371/JOURNAL.PGEN.1010717

Navarro A, Barton NH (2003) Accumulating postzygotic isolation genes in parapatry: A new twist on chromosomal speciation. Evolution 57:447–459. https://doi.org/10.1111/j.0014-3820.2003.tb01537.x

Nei M, Maruyama T, Wu C (1983) Models of evolution of reproductive isolation. Genetics 103:557–579. https://doi.org/10.1093/genetics/103.3.557

Pardo-Manuel de Villena F, Sapienza C (2001) Female meiosis drives karyotypic evolution in mammals. Genetics 159:1179–1189. https://doi.org/10.1093/GENETICS/159.3.1179

Peona V, Blom MPK, Xu L et al (2021) Identifying the causes and consequences of assembly gaps using a multiplatform

genome assembly of a bird-of-paradise. Mol Ecol Resour 21:263–286. https://doi.org/10.1111/1755-0998.13252

Phadnis N, Orr HA (2009) A single gene causes both male sterility and segregation distortion in Drosophila hybrids. Science 323:376–379. https://doi.org/10.1126/science.1163934

R Core Team (2020) R: A language and environment for statistical computing

Rosin LF, Gil J, Drinnenberg IA, Lei EP (2021) Oligopaint DNA FISH reveals telomere-based meiotic pairing dynamics in the silkworm, Bombyx mori. Plos Genet 17:e1009700. https://doi.org/10.1371/journal.pgen.1009700

Schlötterer C, Tobler R, Kofler R, Nolte V (2014) Sequencing pools of individuals — mining genome-wide polymorphism data without big funding. Nat Rev Genet 15:749–763. https://doi.org/10.1038/nrg3803

Senaratne AP, Muller H, Fryer KA et al (2021) Formation of the CenH3-Deficient Holocentromere in Lepidoptera Avoids Active Chromatin. Curr Biol 31:173-181.e7. https://doi.org/10.1016/J.CUB.2020.09.078

Šíchová J, Voleníková A, Dinca V et al (2015) Dynamic karyotype evolution and unique sex determination systems in Leptidea wood white butterflies Speciation and evolutionary genetics. BMC Evol Biol 15. https://doi.org/10.1186/s12862-015-0375-4

Smith DAS (1976) Evidence for autosomal meiotic drive in the butterfly danaus chrysippus L. Heredity (edinb) 36:139–142. https://doi.org/10.1038/hdy.1976.13

Stewart NB, Ahmed-Braimah YH, Cerne DG, McAllister BF (2019) Female meiotic drive preferentially segregates derived metacentric chromosomes in Drosophila. bioRxiv 638684. https://doi.org/10.1101/638684

Suomalainen E, Cook LM, Turner JRG (1973) Achiasmatic oogenesis in the Heliconiine butterflies. Hereditas 74:302–304. https://doi.org/10.1111/j.1601-5223.1973.tb01134.x

Talla V, Johansson A, Dincă V et al (2019) Lack of gene flow: narrow and dispersed differentiation islands in a triplet of Leptidea butterfly species. Mol Ecol 28:3756–3770. https://doi.org/10.1111/mec.15188

Tao Y, Hartl DL, Laurie CC (2001) Sex-ratio segregation distortion associated with reproductive isolation in Drosophila. Proc Natl Acad Sci U S A 98:13183–13188. https://doi.org/10.1073/PNAS.231478798/ASSET/1657961B-AECE-44EB-A2B5-0A3DECAEC5C8/ASSETS/GRAPHIC/PQ2314787004.JPEG

Templeton AR (1981) Mechanisms of speciation - a population genetic approach. Annu Rev Ecol Syst 12:23–48. https://doi.org/10.1146/annurev.es.12.110181.000323

Turner JRG, Sheppard PM (1975) Absence of crossing-over in female butterflies (Heliconius). Heredity (edinb) 34:265–269

Vila R, Lukhtanov VA, Talavera G et al (2010) How common are dot-like distributions? Taxonomical oversplitting in western European Agrodiaetus (Lepidoptera: Lycaenidae) revealed by chromosomal and molecular markers. Biol J Linn Soc 101:130–154. https://doi.org/10.1111/J.1095-8312.2010.01481.X

Walsh JB (1982) Rate of accumulation of reproductive isolation by chromosome rearrangements. Am Nat 120:510–532. https://doi.org/10.1086/284008

Wei KHC, Reddy HM, Rathnam C et al (2017) A pooled sequencing approach identifies a candidate meiotic driver in Drosophila. Genetics 206:451–465. https://doi.org/10.1534/GENETICS.116.197335/-/DC1

White MJD (1968) Models of speciation. Science 159:1065–1070. https://doi.org/10.1126/science.159.3819.1065

Wright CJ, Stevens L, Mackintosh A et al (2024) Comparative genomics reveals the dynamics of chromosome evolution in Lepidoptera. Nat Ecol Evol 8:777–790. https://doi.org/10.1038/s41559-024-02329-4

Wu T, Lane SIR, Morgan SL, Jones KT (2018) Spindle tubulin and MTOC asymmetries may explain meiotic drive in oocytes. Nat Commun 91(9):1–11. https://doi.org/10.1038/s41467-018-05338-7

Publisher's Note   Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.