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# Reproductive isolation in a nascent species pair is associated with aneuploidy in hybrid offspring

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Speciation may occur when the genomes of two populations accumulate genetic incompatibilities and/or chromosomal rearrangements that prevent inter-breeding in nature. Chromosome stability is critical for survival and faithful transmission of the genome, and hybridization can compromise this. However, the role of chromosomal stability on hybrid incompatibilities has rarely been tested in recently diverged populations. Here, we test for chromosomal instability in hybrids between nascent species, the 'dwarf' and 'normal' lake whitefish (*Coregonus clupeaformis*). We examined chromosomes in pure embryos, and healthy and malformed backcross embryos. While pure individuals displayed chromosome numbers corresponding to the expected diploid number ($2n = 80$), healthy backcrosses showed evidence of mitotic instability through an increased variance of chromosome numbers within an individual. In malformed backcrosses, extensive aneuploidy corresponding to multiples of the haploid number ($1n = 40$, $2n = 80$, $3n = 120$) was found, suggesting meiotic breakdown in their $F_1$ parent. However, no detectable chromosome rearrangements between parental forms were identified. Genomic instability through aneuploidy thus appears to contribute to reproductive isolation between dwarf and normal lake whitefish, despite their very recent divergence (approx. 15–20 000 generations). Our data suggest that genetic incompatibilities may accumulate early during speciation and limit hybridization between nascent species.

## 1. Introduction

A fundamental goal in modern evolutionary biology is to characterize the barriers that promote and secure divergence between nascent species, thus resulting in reproductive isolation and ultimately speciation [1,2]. Pre-zygotic barriers have been shown to contribute more to total reproductive isolation than post-zygotic barriers between sympatric species pairs [3]. However, intrinsic post-zygotic reproductive barriers are thought to be permanent and contribute significantly to speciation in an irreversible fashion [4,5]. Among post-zygotic reproductive barriers, it is now clear that nucleotide divergence and genome re-organization through chromosomal rearrangements are intrinsically associated [6–9]. Although it is challenging to study these processes in non-model species, unravelling how nucleotide and chromosomal divergence accumulate and interact to lead to reproductive isolation is crucial to the understanding of speciation.

While the cytogenetic impact of interspecific hybridization has long been studied, it has only rarely been investigated between nascent species [10–12]. One notable exception is the study of chromosomal races in the house mouse (*Mus musculus* complex [13,14]), including a recent study showing that chromosome asynapsis between subspecies hybrids is responsible for infertility [15]. Given the scarcity of studies examining lineages in early stages of divergence, it is hard to draw any conclusions regarding the cytogenetic impact of hybridization and its role in reproductive isolation, and how it varies across

taxa or the divergence time of the system under scrutiny. Indeed, as divergence time increases, the initial genetic changes leading to reproductive isolation will be mixed with subsequent genetic changes that accumulate over time [16]. This will make it more difficult to detect causative mutations leading to reproductive isolation, including the role of chromosomal stability in early speciation [8]. To decipher the initial causes of divergence, and specifically the role of chromosome changes, it is thus necessary to look at the very first stages of speciation.

The geographical and ecological contexts under which divergence has occurred in the lake whitefish (*Coregonus clupeaformis*) system are well understood, thus making it an ideal system in which to study the early stages of speciation. The Acadian and Atlantic lake whitefish lineages were geographically separated approximately 60 000 YBP or approximately 12 000–15 000 generations ago [17,18], during which time, according to the Bateson–Dobzhansky–Muller model (BDM), they could freely accumulate genetic incompatibilities [5,19,20]. This geographical isolation was followed by secondary contact in newly formed lakes after the Laurentian ice sheet retreated approximately 12 000 YBP (approx. 3–4000 generations ago). Following secondary contact, the Acadian lineage evolved repeatedly by character displacement into a 'dwarf' limnetic form while the Atlantic lineage maintained the 'normal' benthic form [17,21]. Gene flow between these sympatric nascent species is still possible [22,23] despite the existence of hybrid incompatibilities leading to a dramatic reduction in embryonic survival in first and second generation hybrids [24–26]. This mortality is associated with the appearance of a 'malformed', slow-growing phenotype in approximately 30–50% of backcross individuals, with the remaining embryos developing normally ('healthy' phenotype) [26]. Consistent with predictions from a BDM model integrating transcriptional data [27], previous studies have documented a much higher variance in gene expression in malformed backcrosses compared with parental forms [26,28]. Transposable elements are also reactivated in both healthy adult backcrosses and malformed backcross embryos, potentially leading to genome instability [28,29]. While earlier studies suggested a primary role for gene expression dysregulation in the appearance of this malformed phenotype [26,28], the molecular basis remains unclear.

Importantly, mixed geographical modes of divergence (i.e. allopatry followed by secondary contact) are predicted to favour chromosome rearrangements between diverging lineages [30]. These chromosome changes can lead to chromosomal incompatibilities in hybrids, either because they will result in unbalanced gametes or disrupt meiosis [12]. Accumulating evidence shows that genetic and chromosomal incompatibilities among species lead to dysregulation involving gene expression, transposable element reactivation and epigenetic inconsistencies in hybrids, all of which also affect genome stability [31,32]. Intriguingly, many aneuploidy events (genome instability in the form of unbalanced segregation of chromosomes) in metazoans lead to similar phenotypes involving significant growth delays combined with malformations [33,34]. Hence, the fact that this malformed phenotype occurs only in post-$F_1$ lake whitefish hybrids, combined with extensive transcriptional dysregulation in backcrosses raises the question of whether aneuploidy might occur in the hybrid progeny of lake whitefish.

In this context, our goal was to test the hypothesis that genomic instability in the form of aneuploidy accompanies hybrid breakdown in the backcross progeny of dwarf and normal lake whitefish. We directly tested if 'healthy' and 'malformed' backcrosses display higher chromosomal instability compared with dwarf and normal lake whitefish by examining embryonic metaphase chromosomes. We reasoned that increased intra-individual variance in chromosome numbers would indicate increased mitotic instability, while increased inter-individual variance would be consistent with meiotic breakdown [35]. As predicted, we found that healthy backcrosses display higher chromosomal instability compared with pure embryos, and this effect is amplified in malformed backcrosses. Moreover, we found haploid, diploid and triploid individuals among malformed backcrosses, suggesting meiotic breakdown in their $F_1$ parents. Yet, conventional karyotyping of the parental forms did not reveal any chromosomal rearrangements. Thus, chromosomal instability occurred in hybrids despite the absence of any obvious chromosomal rearrangements between dwarf and normal genomes. Our results thus support the hypothesis that chromosomal instability in hybrids, possibly resulting from the accumulation of minute chromosomal or genetic divergence in allopatry, represents a strong post-zygotic reproductive barrier in this nascent species complex.

## 2. Material and methods

### (a) Crosses and sampling

Dwarf lake whitefish (Acadian lineage) were caught on their spawning grounds in a tributary draining into Lake Témiscouata (47°41′ N, 68°47′ W) and normal lake whitefish (Atlantic lineage) were caught near Lake Aylmer (45°54′ N, 71°20′ W) during autumn 2011. Sperm and eggs were collected in the field and brought to the laboratory for artificial fertilization. Additionally, two laboratory-reared mature $F_1$-hybrid males (produced from a dwarf mother from Lake Témiscouata and a normal father from Lake Aylmer) from a previous study were used [36]. In total, eight partially half-sib backcross families (i.e. four half-sib families from the same $F_1$-hybrid father and four half-sib families from the other $F_1$-hybrid father) were produced and used in this study. Owing to the limited availability of sexually mature fish, it was impossible to create all complementary crosses (i.e. normal mother × dwarf father). However, previous work has documented similar mortality for both types of crosses [24,25]. Moreover, the malformed phenotype also occurs in the reciprocal backcross (i.e. with a $F_1$-hybrid female [26]). A complete description of the embryos sampled in this study can be found in table 1. It should be noted that the malformed phenotype was found in all backcross families, although only a subset was sampled.

All eggs were incubated in the same slowly flowing water system (4.5–5.5°C) and reared in a common environment at the LARSA (Laboratoire de recherche en sciences aquatiques, Université Laval).

### (b) Chromosome preparation and microscopy

Healthy (pure dwarf, pure normal and backcross) and malformed (only found among backcrosses) individuals were sampled. It should be noted that the malformed phenotype segregates within all backcross families. As previously documented within the same long-term research programme, the malformed phenotype is easily identified by the strong deformities seen, including a curved tail and no visually detectable heartbeat. Malformed individuals still display characteristics of the phylotypic

**Table 1.** Individuals sampled in this study. The family name reflects the number of the parent and the direction of the cross (female × male). N, normal; D, dwarf.

| type | family | *n* healthy | *n* malformed | total |
|---|---|---|---|---|
| N × N | N14 × N1 | 2 | 0 | 10 |
| | N15 × N7 | 2 | 0 | |
| | N17 × N8 | 2 | 0 | |
| | N18 × N9 | 1 | 0 | |
| | N19 × N13 | 3 | 0 | |
| D × D | D55 × D79 | 4 | 0 | 11 |
| | D57 × D80 | 3 | 0 | |
| | D58 × D78 | 1 | 0 | |
| | D59 × D75 | 3 | 0 | |
| backcross | D63 × F1–2 | 3 | 3 | 20 |
| | D64 × F1–1 | 2 | 0 | |
| | D68 × F1–2 | 1 | 2 | |
| | D72 × F1–2 | 0 | 1 | |
| | N17 × F1–2 | 1 | 0 | |
| | N18 × F1–1 | 0 | 2 | |
| | N25 × F1–1 | 3 | 0 | |
| | N28 × F1–1 | 0 | 2 | |
| total | | 31 | 10 | 41 |

stage, including eye and dorsal line pigmentation, but still do not resemble any earlier stage of development in normally developing embryos (see [26] for more details).

Chromosome suspensions from embryos were prepared following a previously published method [37] using early-eyed stage embryos (approx. 150–180 degree-days, i.e. 30–36 days of development at 5°C). Chromosome suspensions from four wild dwarf individuals (from Lake Témiscouata, two males and two females) and four laboratory-reared normal individuals (from Lake Aylmer, undetermined sex) were prepared using leucocyte culture as described elsewhere [38]. Unfortunately, these individuals could not be sexed, as there is currently no sex marker available for *Coregonus* [39]. In addition, we did not detect a heteromorphic sex chromosome in either dwarf or normal individuals, a common situation in salmonids [40]. Admittedly, it cannot be determined whether we karyotyped normal males, normal females or a mix of them.

Metaphase chromosomes were stained with Giemsa–Romanowski dye (pH 6.8–7.0, Dr Kulich Pharma, Hradec Králové, Czech Republic) following standard protocols and examined using a Provis AX70 Olympus microscope. Images were captured with a CCD camera (DP30W Olympus). A total of 402 metaphase spreads from 41 embryos were examined, in addition to 64 metaphases from the eight adult fish (table 1). With the exception of one embryo for which only four observations could be made, at least five metaphases were examined per embryo, with an average of 9.8 observations per individual (table 2). In adults, eight metaphases were karyotyped per individual.

## (c) Statistical analyses

All statistical analyses were performed using R v. 2.15.1 [41]. We first tested whether intra-individual variance of chromosome counts was dependent on the experimental group (i.e. pure dwarf, pure normal, backcross healthy or backcross malformed). We thus performed an ANOVA on log-transformed individual coefficient of variation of chromosome numbers. Coefficients of variation were used to control for the apparent correlation between chromosome number variance and ploidy level. The Tukey HSD test was then used to identify the comparisons responsible for the significant differences among groups.

We then tested the hypothesis that the variance in median chromosome numbers per individual is significantly different between groups. Specifically, we wanted to know if the variance in median chromosome counts per individual was higher in malformed backcrosses. We applied the Fligner–Kelleen test for homogeneity of variance on median chromosome counts per individual, first on all groups, and then using pairwise comparisons among all groups. A false-discovery rate (FDR) correction was applied to *p*-values using the function *p.adjust*.

# 3. Results

Adult Giemsa-stained karyotypes corresponded to previously described karyotypes [42]. Both have a diploid chromosome number of $2n = 80$, including 10 meta-/sub-metacentric chromosome pairs and 30 acrocentric chromosome pairs of gradually decreasing size, with the exception of one distinguishable large pair. No obvious differences were detected between the karyotypes of the two forms (figure 1*a*,*b*).

Summary statistics of chromosome number per group can be found in table 2. The complete summary statistics of chromosome number per individual are in electronic supplementary material, table S1. In pure dwarf and normal embryos, counts were centred on the expected diploid number ($2n = 80$, figure 2*a*,*b*; [42]). Dwarf embryos had a mean of $81.7 \pm 11.7$ chromosomes/metaphase and a median of 79 chromosomes/metaphase, whereas normal embryos had a mean of $78.3 \pm 5.6$ chromosomes/metaphase and a median of 79 chromosomes/metaphase (table 2). Among pure embryos, no counts exceeded 86 chromosomes, with the exception of a single suspected triploid dwarf individual (figure 3*a*, mean = $108.1 \pm 13.5$ chromosomes/metaphase, median = 111.5 chromosomes/metaphase). Pure embryos displayed some variance around the diploid number ($2n = 80$, figures 2*a*,*b* and 3), which was expected as chromosome suspensions from embryos are more difficult to spread than those from other tissues, and therefore more difficult to count ([43]; figure 1).

In healthy backcrosses (figure 2*c*), counts were also centred on $2n = 80$ (median = 78 chromosomes/metaphase) but with a lower mean ($73.7 \pm 13.3$ chromosomes/metaphase) compared with pure embryos (table 2). Metaphases with 20–86 chromosomes were found, but all of these individuals seemed diploid (with a median chromosome number close to 80), although with increased variance in chromosome number.

In sharp contrast, a clear tri-modal distribution was found in malformed backcrosses (figures 2*d* and 3*a*), with chromosome numbers concentrated around multiples of the haploid number ($1n = 40$, $2n = 80$, $3n = 120$). The mean chromosome number was lower than all other groups (mean = $76.0 \pm 34.7$ chromosomes/metaphase), while the median was equal to healthy backcrosses (median = 78 chromosomes/metaphase, table 2). Malformed backcrosses could be separated according to their ploidy (figures 1*c*–*e* and 3*a*). Three malformed backcross individuals were clear

**Table 2.** Summary statistics of chromosome number per cross-type and group. N, normal; D, dwarf; BC, backcross.

| group | *n* individuals | mean | s.d. | median | *n* metaphases |
|---|---|---|---|---|---|
| N × N | 10 | 78.3 | 5.6 | 79 | 102 |
| D × D | 11 | 81.7 | 11.7 | 79 | 95 |
| BC healthy | 10 | 73.7 | 13.3 | 78 | 103 |
| BC malformed | 10 | 76.0 | 34.7 | 78 | 102 |

haploids ($1n = 40$), one individual was a triploid ($3n = 120$) and one individual was almost tetraploid (figure 3*e*, mean = 139 ± 20.8 chromosomes/metaphase, median = 145 chromosomes/metaphase). Metaphases with as few as 32 chromosomes to as many as 158 chromosomes were found in malformed backcrosses. Chromosome fragments were also found in malformed backcrosses, although these were relatively rare (figure 1*e*, arrowheads).

An ANOVA testing for differences in the intra-individual coefficient of variation of chromosome counts revealed a significant difference among groups ($F_{3,36} = 4.911$, $p = 0.0058$). There was a significant difference between healthy backcrosses and pure normal and dwarf embryos (Tukey HSD test, $p \leq 0.05$), but no comparison involving malformed backcrosses was significant (figure 3*b*). This is because there were three haploids among malformed backcrosses, which had very small variance of chromosome numbers. In addition, we found a significant difference in the variance of the median chromosome number among groups (Fligner–Killeen test, $\chi^2 = 12.0222$, d.f. = 3, $p < 2.20 \times 10^{-16}$). The variance of median chromosome number in malformed backcrosses was significantly different from pure normal, pure dwarf and healthy backcrosses, after correction for multiple testing (figure 3*c*, Fligner–Killeen test, FDR < 0.05, $p \leq 0.05$). Complete statistical analyses can be found in electronic supplementary material, table S4.

## 4. Discussion

In this study, we investigated the role of chromosomal instability in reproductive isolation between nascent lake whitefish species pairs by measuring the chromosome numbers of normal, dwarf, and healthy and malformed backcrosses. Increased intra-individual variance of chromosome number was found in healthy backcrosses. This strongly supports the hypothesis of mitotic chromosome segregation problems, resulting in extra or missing chromosomes after mitotic cell division [35]. However, malformed backcrosses did not display evidence for mitotic chromosome instability compared with pure embryos. This is likely because three stable haploid malformed backcrosses were sampled, thus reducing the variance within the group. Even more strikingly, higher inter-individual variance was found in malformed backcrosses than any other group, i.e. extensive aneuploidy, with an extra or missing haploid complement in the majority of individuals. This result is consistent with meiotic non-disjunction in their $F_1$-hybrid parent [35]. Yet, karyotypes from parental forms did not reveal any obvious differences at the whole-chromosome level. This suggests that aneuploidy in hybrids is caused by minute sub-chromosomal incompatibilities or genetic incompatibilities acting through mitotic and meiotic mechanisms. The accumulation of these incompatibilities may have been facilitated by the geographical isolation between the two pure forms for approximately 12 000–15 000 generations. Clearly, such incompatibilities cause substantial reproductive isolation between lake whitefish lineages, as 30–50% of post-$F_1$-hybrids are malformed and die during their early development [25,26]. Our results are especially noteworthy considering the very young age of these lineages on an evolutionary timescale [18].

Importantly, our results were collected from eight partially half-sib backcross families, arguing that these results are not only due to a 'family effect', but apply more generally to these populations. Moreover, the malformed phenotype associated with aneuploidy was observed in two different cohorts (crosses from [26] and this study). Finally, the malformed phenotype has also been observed in post-$F_2$ hybrids (A.-M.D.-C. and L.B. 2011, 2012, unpublished data). These independent observations strongly support the hypothesis of segregating sub-chromosomal or genetic incompatibilities between lake whitefish lineages leading to aneuploidy in their hybrid progeny, and reproductive isolation.

We note that this extensive aneuploidy would have been very difficult to interpret or even detect from whole genome sequence data alone, stressing the importance of cytogenetics in the post-genomic era. While these approaches have been largely neglected since the advent of modern sequencing techniques, we have shown here that they provide key information regarding genome organization and stability that are difficult to detect from sequence data.

### (a) Potential mechanisms underlying chromosome segregation breakdown

We predicted that lake whitefish hybrids would display higher chromosomal instability compared with pure parental forms. Our data support this prediction and here, we discuss four, non-mutually exclusive, candidate mechanisms that are potentially responsible for chromosomal segregation breakdown, namely (i) chromosomal rearrangements, (ii) the mismatch repair (MMR) pathway, (iii) centromere divergence and (iv) heterochromatin decondensation.

The most parsimonious explanation for both mitotic and meiotic breakdown in backcrosses is that significant chromosomal rearrangements have occurred between the Atlantic and Acadian lake whitefish lineages, thus interfering with proper meiotic and mitotic chromosomal segregation [35]. However, karyotyping suggests that this is not the case, at least at the whole-chromosome scale as both karyotypes are essentially the same (figure 1*a*,*b*). Yet, our results cannot rule out the possibility that more subtle changes at the

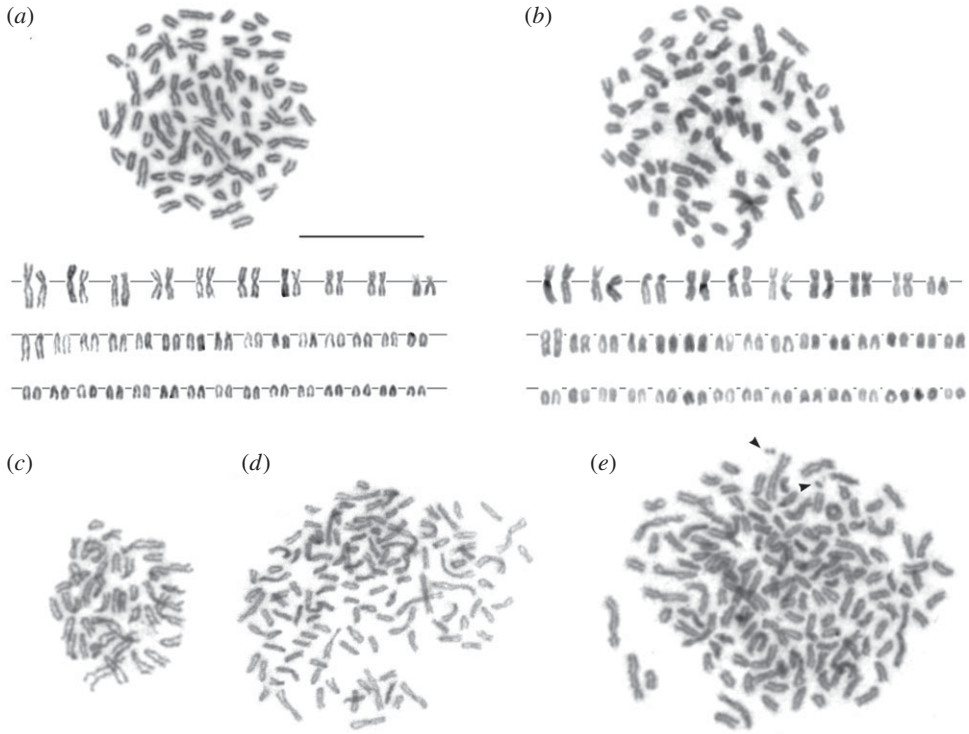


**Figure 1.** Karyotypes of pure parental forms and abnormal metaphases of malformed backcrosses. Pure karyotypes are composed of 10 metacentric pairs and 30 acrocentric pairs of decreasing size. (*a*) Normal individual from Lake Aylmer. (*b*) Dwarf individual from Lake Témiscouata. (*c*) Haploid metaphase from a malformed backcross. (*d*) Triploid metaphase from a malformed backcross. (*e*) Nearly tetraploid metaphase from a malformed backcross. Arrowheads denote chromosome fragments. Scale bar, 10 µm.

sub-chromosomal level might be involved, including heterochromatin and rDNA genes additions/deletions, with potential consequences for gene expression regulation. It is noteworthy that such sub-chromosomal changes have been recently detected in another *Coregonus* species pair from Europe, where no major karyotypic differences were found [44].

Alternatively, nucleotide divergence among ecotypes may prevent the proper functioning of DNA repair pathways, such as the highly conserved MMR DNA repair pathway [45], and result in meiotic breakdown. Indeed, this mechanism is responsible for induced aneuploidy between incipient species of yeast (*Saccharomyces*, [46]). Meiotic crossovers are critical for balanced chromosome segregation in meiosis as they maintain a tight connection between homologous chromosomes during meiosis I. When divergent chromosomes are combined in yeast hybrids, the MMR pathway prevents these crossovers, thus resulting in aneuploid progeny. Thus, the MMR pathway may underlie the meiotic breakdown in lake whitefish $F_1$-hybrids if nucleotide divergence is high enough in regions targeted by the meiotic recombination machinery. However, this mechanism does not provide an explanation for mitotic breakdown in healthy backcrosses.

A third candidate mechanism leading to increased chromosomal instability in hybrids was originally proposed by Henikoff *et al.* [47] based on the observation of concerted, rapid evolution of centromeres and their associated proteins. Centromeres are defined by repetitive sequences, including transposable elements. Centromeres are thus rapidly evolving due to their labile nature, despite their highly conserved and critical role in chromosome segregation. This could lead to chromosomal incompatibilities, even between allopatric populations of the same species [9,47]. Hence, it is plausible that aneuploidy in lake whitefish backcrosses may result from the disruption of the chromosome segregation machinery via centromere incompatibilities.

A fourth possibility is that aneuploidy results from heterochromatin decondensation due to mis-regulation in lake whitefish hybrids, which could significantly affect chromosome segregation in both mitosis and meiosis [48]. Indeed, accumulating studies support a role for heterochromatin regulation and associated proteins in reproductive isolation [31,49–51]. Therefore, heterochromatin deregulation in lake whitefish hybrids could also disrupt mitotic and meiotic chromosome pairing, inducing aneuploidy in backcrosses.

We cannot yet conclusively state which of these molecular mechanisms is responsible for the aneuploidy in lake whitefish backcrosses. However, the heterochromatin decondensation hypothesis is especially promising, as previous work in our system has found a massive reactivation of both transposable elements and non-coding RNAs in malformed backcrosses, consistent with heterochromatin disruption [26,28]. Also, we previously found that the Gene Ontology category 'chromosome condensation' was enriched among genes differentially expressed between dwarf and normal embryos, suggesting divergence in the regulation of chromosome compaction [28]. We also note that the reactivation of transposable elements may lead to aneuploidy via chromosomal rearrangements (mechanism 1). Further studies looking specifically at sub-chromosomal structure and heterochromatin regulation in dwarf and normal lake whitefish, as well as their hybrids, will help to disentangle these potentially non-mutually exclusive mechanisms.

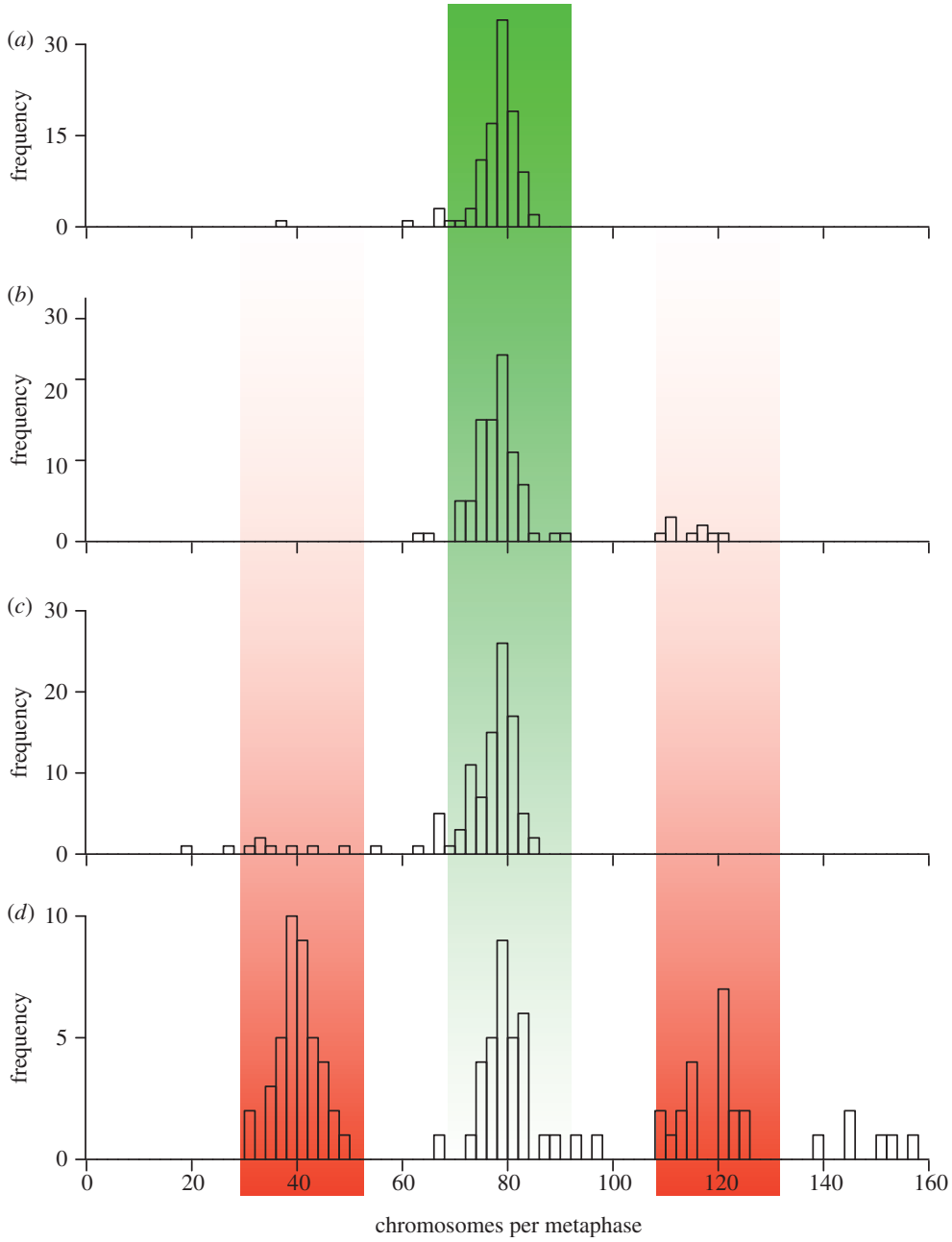


**Figure 2.** Meiotic breakdown in malformed backcrosses reflected by the analysis of mitotic chromosomes. Histogram showing the distribution of chromosome counts in lake whitefish embryos. (*a*) Normal fish ($n = 10$), (*b*) dwarf fish ($n = 11$), (*c*) healthy backcrosses ($n = 10$) and (*d*) malformed backcrosses ($n = 10$). (Online version in colour.)

## (b) Development canalization and 'aneuploidy syndrome'

The fact that healthy backcrosses appear to develop normally and can eventually reproduce (A.-M.D.-C. and L.B. 2011, 2012, unpublished data) despite mitotic instability questions how such intra-individual variation is buffered through development. Indeed, Waddington [52] elegantly suggested that developmental pathways are under strong selective pressure (or canalized), thus buffering for genetic and environmental variations. Hence, the phenotype of healthy backcrosses appears canalized despite higher chromosomal variation compared with pure embryos.

However, this canalization is broken down in malformed backcrosses. The malformed phenotype occurs in conjunction with more variable cytogenetic backgrounds and also in putative diploid individuals (figure 3*a*). Moreover, malformed backcrosses did not show statistically significant mitotic instability ($p = 0.15$ versus dwarf embryos and $p = 0.09$ versus normal embryos, Tukey HSD post hoc test; electronic supplementary material, table S3). As explained above, this is because three haploid malformed backcrosses had a much smaller variance of chromosome number (within an individual) than other malformed backcrosses (figure 3*a*; electronic supplementary material, table S1). These haploid individuals may suffer from this 'aneuploidy syndrome', but not hybrid incompatibilities *per se* as they bear only one genome (likely the pure maternal).

How can one explain this consistent malformed phenotype despite high cytogenetic and transcriptional variability [26,28]? Lindsley *et al*. [33] found that different types of hyperploidy in *Drosophila* resulted in a common phenotype combining rough eyes, abnormal wings, bristle and abdomen, which they described as a 'hyperploidy syndrome'.

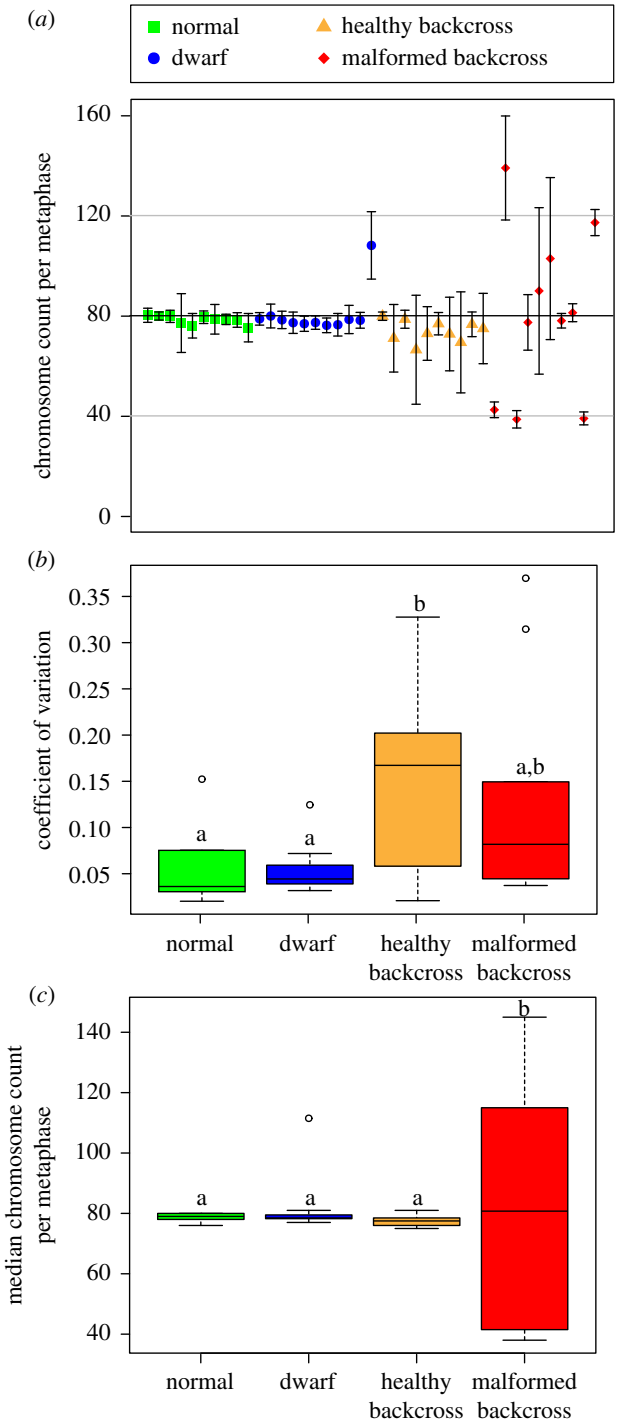


**Figure 3.** Mitotic and meiotic chromosomal instability occurs in backcrosses based on the analysis of mitotic chromosomes. (*a*) Mean chromosome counts and standard deviation per individual for each group. (*b*) Boxplot showing the distribution of individual coefficient of variation of chromosome number per group. Different letters indicate statistically significant differences (Tukey HSD test, $p \leq 0.05$). (*c*) Boxplot showing the distribution of median chromosome number per group. Different letters indicate statistically significant differences (Fligner–Killeen test, FDR $< 0.05$, $p \leq 0.05$). (Online version in colour.)

A recent study also found a consistent transcriptional profile within species that was independent of the specific chromosome aberration investigated [53]. In general, many organisms for which the aneuploidy effect has been studied were found to display developmental abnormalities, in addition to a transcriptional signature involving protein synthesis, inflammatory and stress responses [34,54]. Not surprisingly, this signature was also found in lake whitefish malformed backcrosses [28], in addition to a downregulation of essential developmental genes [26].

Aneuploidy may contribute to the mis-regulated transcriptional landscapes previously described in lake whitefish backcross embryos; alternatively, transcriptional mis-regulation in hybrids may lead to aneuploidy. While the causal relationship remains difficult to establish, it appears that the malformed phenotype and associated transcriptional response that we identified in lake whitefish hybrids mirror what has been found in other organisms. Developmental canalization breakdown in malformed backcrosses is thus associated with an 'aneuploidy syndrome' involving increased chromosomal instability and a distinctive transcriptional response.

## (c) Implications for the study of speciation

Although cytogenetic studies looking at early diverging lineages are scarce, reproductive isolation through chromosomal instability has been observed in hybrids across several taxa. In yeast (*Saccharomyces paradoxus*), a recent study found that chromosomal differences lead to chromosomal instability in the progeny of diverging strains [55]. Importantly, the divergence of these strains occurred under a similar biogeographical context as the lake whitefish, i.e. a phase of geographical isolation followed by recent secondary contact between lineages. This further supports our interpretation that the conditions under which divergence occurred in lake whitefish have facilitated the accumulation of incompatibilities, which may be either of chromosomal or genetic nature, leading to chromosomal instability in hybrids. In the house mouse (*Mus musculus domesticus*), numerous studies have clearly shown that hybridization between certain chromosomal races leads to chromosomal mis-segregation and hence reduction in litter size [14,56]. Combined with our results, these studies suggest that chromosomal instability can occur in the hybrid progeny of early diverging lineages across a broad range of taxa.

However, it should be noted that, divergence time among these lineages is much greater than for the lake whitefish, given that mouse races have diverged several hundred thousands to million years ago [57] and that yeast produces multiple generations per year. Moreover, it should be stressed that the chromosomal instability we have documented appears to occur in the absence of detectable chromosomal rearrangement. To our knowledge, our study is thus the first to investigate the cytogenetic impact of hybridization among such recently diverged lineages (approx. 12–15 000 generations), at least in vertebrates. There are few, if any, examples of such striking incompatibilities in lineages as young as the lake whitefish, and it is possible that this is only because the cytogenetic consequences of hybridization have been overlooked.

As a consequence, chromosomal speciation models, including the cytogenetic impact of hybridization, have been somewhat neglected in the past decade. This is also due to the combination of the presumed small involvement of chromosome rearrangements to early speciation stages (with the exception of inversions, e.g. [6,7,58]) and theoretical issues concerning the fixation of strongly underdominant chromosomal rearrangements [8]. However, the conditions promoting the fixation of new chromosomal rearrangements

were present in the lake whitefish system, including: a mixed geographical mode of divergence [17], small effective population size ($Ne \sim 1000$, [59]), geographical isolation of lineages [60] and possibly meiotic drive [61]. Unfortunately, it is not yet possible to determine whether the chromosomal instability observed in lake whitefish hybrids is the result of genetic or chromosomal incompatibilities, and hence a case of chromosomal speciation. Yet, both genetic and subtle chromosomal changes may be involved, and future research should help to disentangle these alternative hypotheses.

Our results are critical to the understanding of how reproductive isolation has emerged in the lake whitefish system, and other nascent species. We show that genomic instability, through aneuploidy, transcriptional dysregulation and transposable reactivation, can interact and efficiently limit hybridization early in the divergence process, and thus contribute to speciation. Future work looking at systems where conditions promoting the appearance and fixation of chromosome rearrangements are found will help to draw conclusions regarding the generality of our observations.

Ethics statement. The Laval University animal care committee (CPAUL) revised and approved all experimental procedures (Protocol 82178).

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