A reassessment of explanations for discordant introgressions of mitochondrial and nuclear genomes

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Hybridization is increasingly recognized as a significant evolutionary process, in particular because it can lead to introgression of genes from one species to another. A striking pattern of discordance in the amount of introgression between mitochondrial and nuclear markers exists such that substantial mitochondrial introgression is often found in combination with no or little nuclear introgression. Multiple mechanisms have been proposed to explain this discordance, including positive selection for introgressing mitochondrial variants, several types of sex-biases, drift, negative selection against introgression in the nuclear genome, and spatial expansion. Most of these hypotheses are verbal, and have not been quantitatively evaluated so far. We use individual-based, multilocus, computer simulations of secondary contact under a wide range of demographic and genetic scenarios to evaluate the ability of the different mechanisms to produce discordant introgression. Sex-biases and spatial expansions fail to produce substantial mito-nuclear discordance. Drift and nuclear selection can produce strong discordance, but only under a limited range of conditions. In contrast, selection on the mitochondrial genome produces strong discordance, particularly when dispersal rates are low. However, commonly used statistical tests have little power to detect this selection. Altogether, these results dismiss several popular hypotheses, and provide support for adaptive mitochondrial introgression.

KEY WORDS: Gene flow, individual-based simulations, introgressive hybridization, isolation by distance, mitonuclear discordance, secondary contact.

Inter-specific hybridization and introgression of genes between taxa, previously seen as rare events that typically lead to evolutionary dead-ends (Mayr 1963), are now known to be relatively common in many plant and animal groups (Grant and Grant 1992; Chan and Levin 2005; Mallet 2005). Introgression challenges many methods of taxonomic, demographic, or historical infer-

ence from genetic data by introducing a mosaic of evolutionary histories across the genome (Funk and Omland 2003).

One of the most striking patterns of introgression is mitonuclear discordance: a significant difference in the degree of introgression observed in mitochondrial and nuclear markers (Toews and Brelsford 2012). Mito-nuclear discordance has been observed in a wide range of animal species: for example mammals (Roca et al. 2005; Berthier et al. 2006; Melo-Ferreira et al. 2009; Hedrick 2010; Good et al. 2015), birds (Irwin et al. 2009; Milá et al. 2011), reptiles (Renoult et al. 2009), actinopterygii (Willis et al. 2014), amphibians (Veith et al. 2012; Zieliński et al. 2013; Lee-Yaw et al. 2014), butterflies (Gompert et al. 2008; Zakharov et al. 2009). Mito-nuclear discordance seems common: a random survey of articles published in *Molecular Ecology* on the topic found mito-nuclear discordance in 11 out of 61 studies (Toews and Brelsford 2012). Moreover, the discordance is often geographically extensive and not limited to the current zone of contact between two taxa.

In the most extreme, a taxon's original mitochondrial lineage can be nearly completely replaced by the introgressing mitochondrial genome, which is said to be "captured," with no or little evidence for concomitant nuclear introgression (Berthier et al. 2006; Hedrick 2010; Zieliński et al. 2013; Good et al. 2015). In this article, we refer to extreme cases of mito-nuclear discordance, where all or nearly all individuals of a species harbor the mitochondrial DNA (mtDNA) of the other species, but with very little nuclear introgression as "massively discordant mitochondrial introgression (MDMI)." Under a neutral model, the probability of fixation of an introgressed allele is its initial frequency, for nuclear and mitochondrial markers both. Therefore it is remarkable that mitochondrial DNA seems prone to reach fixation in a wide variety of taxa while the other loci undergo only limited introgression. Several mechanisms have been proposed to explain MDMI.

A primary hypothesis is that there is positive selection for the introgressing mitochondrial genome. This would occur if mitochondria from the introgressing taxon have a fitness advantage over the mitochondria of the introgressed taxon. Under this scenario, mitochondrial capture can occur with little nuclear introgression (Ballard and Whitlock 2004; Fitzpatrick et al. 2009). Variation in mtDNA has long been assumed to be neutral (e.g., Mitton 1997, p19), thus weakening the credibility of the selective hypothesis. The neutrality of mtDNA variation is increasingly questioned (Ballard and Whitlock 2004; Bazin et al. 2006; Meiklejohn et al. 2007; Dowling et al. 2008; Galtier et al. 2009; Heulin et al. 2011), but it still remains a common null-hypothesis. Positive selection has been invoked to explain discordance in introgression include the sharing of a mitochondrial lineage across several Drosophila species (Bachtrog et al. 2006) and mitochondrial functional variation is thought to have played a role in past introgressions within a warbler species (Toews et al. 2014). Two studies of on-going selection of introgressed mitochondrial haplotypes further support the credibility of this hypothesis: (i) In bank voles, introgressed mitochondrial haplotypes are correlated with variation in metabolic rates along a latitudinal gradient in a way that appears adaptive (Boratynski et al. 2011); (ii) In Ambystoma salamanders, mitochondrial haplotypes account for some variation in fitness proxies, along with the nuclear genome (Lee-Yaw et al. 2014). Demonstrating past selective events is often challenging, especially for mtDNA that recombines very rarely and effectively behaves as a single locus (Galtier et al. 2009), and fitness effects of mtDNA variants may not be easily detected in contemporary studies if selection is due to selfish mitochondrial mutations or selfish maternally inherited symbionts (Galtier et al. 2009). These limits could explain the paucity of empirical evidence for adaptive introgression of mtDNA in natural populations, and adaptive introgression of mtDNA could be more widespread than the literature currently suggests.

Alternatively, there are several hypotheses that do not involve positive selection on mtDNA, but other processes that can lead to differential introgression. In the rest of the manuscript we will refer to these hypotheses as "mt-neutral." Three involve some kind of sex-related asymmetry in interspecific gene flow. First, if females of taxon 1 preferentially select mates of their own taxon while females of taxon 2 mate randomly, mitochondria of taxon 2 will be overrepresented among hybrids. The repetition of such asymmetric crosses over several generations has been proposed to gradually lead to mito-nuclear discordance (Wirtz 1999; Roca et al. 2005; Hedrick 2010). Second, sex-differences in hybrid survival, for instance following Haldane's rule (Coyne 1985), has also been proposed as a source of mito-nuclear discordance (Hedrick 2010). Third, asymmetrical introgression could be the result of sex-biased dispersal in two distinct contexts. Introgression of mitochondria could be favored by female-biased dispersal in stable contact zones (Roca et al. 2005) as well as by male-biased dispersal in a context of spatial invasion (Petit and Excoffier 2009). After an invasion with sex-biased dispersal, the genetic composition in the invaded area consists of more local genes (vs invading genes) at loci associated with the least dispersing sex compared to loci associated with the most dispersing sex (Petit and Excoffier 2009). In species with male-biased dispersal, an invasion scenario is therefore compatible with higher introgression for mitochondria than for nuclear loci. However, this does not necessarily mean there is little nuclear introgression. The simulations used to examine the effect of invasion and dispersal on introgression examined a single locus (Excoffier et al. 2009; Petit and Excoffier 2009), and there is no reason to suppose that mitochondria would introgress significantly without substantial nuclear introgression.

While these three sex-biased processes can easily produce a number of individuals with alien mitochondria in a nuclear background almost clear of introgression (except for the W chromosomes in species with heterogametic females), it is not clear how these individuals could transmit their genomic composition to the whole population and produce MDMI. Except under very strict artificial breeding conditions, backcrossed individuals with the discordant mito-nuclear combination should represent only a small fraction of the population. Admittedly, a demographic expansion following introgressive hybridization in a small population could magnify an early effect of drift that would have fixed mtDNA but discarded most of the nuclear genome (Berthier et al. 2006). This theoretically possible explanation can hardly be a general one, because drift alone is not expected to produce such a contrasted pattern frequently. Generally, extensive mtDNA introgression at the population-level requires high levels of gene-flow, which would inevitably increase the frequency of introgressed nuclear genes. In summary, these different hypotheses based on sex-biased life-history traits (dispersal, survival or fecundity of hybrids, or mate choice) share the common weakness that they do not explicitly provide a mechanism for very low nuclear introgression (i.e., few nuclear gene copies of introgressed origin), but simply argue that mitochondrial introgression should be favored compared to nuclear introgression.

An alternative mt-neutral hypothesis (i.e., neutral with respect to mtDNA) involves negative selection on multiple nuclear loci. Provided that post-zygotic isolation between hybridizing species is due to enough nuclear loci spread over the nuclear genome, introgression of nuclear loci could be restricted to low levels while mitochondria could cross the species barrier (Funk and Omland 2003). While intuitively possible, this explanation probably requires intermediate levels of postzygotic isolation (strong isolation would prevent all introgression, low isolation would allow extensive nuclear introgression) and it is unclear whether mtDNA could really escape its original genetic background when the level of postzygotic isolation is strong enough to lock the nuclear genome.

Here, we model secondary contact between two taxa under a wide range of demographic and selective scenarios to identify conditions favoring mito-nuclear discordance. We combine backward coalescence and forward individual-centered algorithms to simulate various demographic and genetic processes simultaneously in a multilocus framework. The forward part of the simulations let us incorporate complex processes that are intractable analytically, while providing a quantitative assessment of complex verbal models, whereas the backward part favors computational efficiency.

We first simulate scenarios in which mean dispersal is high and therefore overall introgression rates are high. With high overall introgression rates, positive selection on mtDNA would not lead to strong mito-nuclear discordance, unless another process is also involved. Indeed, even in the case of a mitochondrial capture (introgression of 100%), the difference between mitochondrial and nuclear introgression would be small if nuclear introgression is high. MDMI would be produced only if there is a decrease of autosomal introgression relative to high mitochondrial introgression. The processes considered in this first part are sex-biased dispersal, sex-biased hybrid survival, asymmetrical crosses between taxa, spatial invasion, negative selection against hybrid and maladapted individuals on multiple autosomal loci, and genetic drift.

We then simulate scenarios in which mean dispersal and introgression rates are low. We find a narrow window of dispersal rate where MDMI can occur, infrequently, due to drift. Below this narrow window of dispersal rates, MDMI could logically be produced by an increase of mitochondrial introgression relative to low autosomal introgression. The processes considered in this second set of simulations are the same three sex-biased mechanisms, spatial invasion, and selection for mitochondrial introgression. Finally, we assess the power of statistics commonly used to detect mitochondrial selection from genetic polymorphism data. We simulated only scenarios corresponding to a verbal hypothesis explaining MDMI. For example, we considered only the effect of sex-biased dispersal in the case of a spatial invasion, because dispersal was the main parameter discussed in the context of an invasion (see Petit and Excoffier 2009). An overview of the scenarios simulated can be found in table 1.

Methods

BIOLOGICAL SCENARIO CONSIDERED

We use spatially explicit, individual-based simulations to model a secondary contact, that is, a hybrid zone between two taxa having diverged in allopatry. Diploid individuals are distributed over a two-dimensional network of demes (subpopulations), each containing 10 adult pairs (Fig. 1). Mating occurs randomly within a deme, except for scenarios with asymmetric crosses between taxa (see below), therefore hybridization rate is controlled through dispersal rate. Every generation, a given proportion of juveniles undergoes spatially limited dispersal (see below) within the network of demes. We assume that the allopatry period has allowed each taxon to adapt to its respective habitat so that individuals of each taxon have a lower fitness when they reproduce in their nonoriginal habitat. In addition, the two diverging taxa have developed genic incompatibilities at the local adaptation loci: they remain interfertile but their hybrids have reduced fitness, irrespective of the habitat. Both types of selection occur on juvenile survival. After dispersal and selection, regulation ensures that deme size is constant.

Divergence has been long enough for the two taxa not to share alleles at any locus, thus allowing straightforward measurement of introgression. Individuals are classified as being pure taxon 1, pure taxon 2 or hybrids, based on genotype at one local adaptation (speciation) locus. An individual homozygous 1|1 at the speciation locus is considered to be taxon 1, and similarly an individual homozygous 2|2 at the speciation locus is considered to be taxon 2. A heterozygous 1|2 individual is considered to be hybrid. This is a proxy for a set of coadapted loci in linkage

		Sex-biases			Selection		
Dispersal	Secondary contact	Dispersal	Hybrid fitness	Mating	Mitochondrial	Nuclear	Single hybridization
High	Stable	х	×	×	×	×	×
	Invasion	×					
Low	Stable	×	×	Х	Х		
	Invasion	×					

Table 1. Overview of the scenarios simulated.

A x indicates scenarios that were simulated, while empty cells indicates scenarios that were not simulated. Scenarios are primarily organized depending on (i) whether the mean dispersal is high (m = 0.1) or low (m = 0.001); and (ii) whether the secondary contact is spatially stable or whether one of the two taxa invades the whole area. Other columns indicate the simulation of sex-biased dispersal; sex-biased hybrid fitness; sex-biased asymmetric mating between taxa; positive mitochondrial selection favoring one of the two mitochondrial lineage; nuclear negative selection opposing the introgression of nuclear genes; and strong genetic drift due to a single hybridization event in a small population. For the latter, note that we simulate high gene flow within taxa, but very low gene flow between taxa.



Figure 1. Deme structure and dispersal patterns. Demes (little circles) are organized on a two dimensional grid, over two habitats (white demes and black demes). Dispersal between demes follows a pattern of isolation by distance and occurs at the juvenile stage, after selection. Dispersal is here represented from only one deme, as arrows whose thickness indicates the relative probability to move to a given deme (distant demes are less likely to be reached). Dispersal rates differ between females and males, but this is not depicted here. Within a deme (big dotted circle), individuals (ellipses) are organized as mating pairs. Initially, all individuals within an habitat have matching local adaptation alleles. Dispersal and hybridization allows the presence of hybrids (i.e., heterozygotes for the local adaptation locus, gray individuals) and maladapted (i.e., homozygotes with the wrong allele for the local adaptation locus, e.g., black individuals in a white deme) individuals. For instance within the deme in focus there are two hybrids, one maladapted individual, and nine adapted individuals.

disequilibrium, that one can think of as defining species (Mallet 1995). Nevertheless, our work does not rely on a particular definition of species and the taxa can instead be seen as evolutionary lineages able to hybridize. Nineteen other local adaptation loci are added for the simulation of some scenarios but they are never used to define taxa. For all the loci under selection, including the speciation locus, only two alleles exist.

In the absence of epistasis or gene transfers, autosomes can be thought of as independent, and therefore redundant, evolutionary units. Therefore, for the nuclear genome we modeled one single autosome, bearing regularly spaced loci. Every second locus is a neutral, mutating, locus, and the other one is a selective locus (controlling local adaptation and fitness reduction in hybrids), not mutating and with only two allelic states. Recombination takes place throughout these alternating loci. Mitochondria also bear a mutating neutral marker and a nonmutating biallelic locus that can be under selection (this selection is among competing mitochondrial lineages, independently of habitat or hybrid-status), and these two loci are perfectly linked. Autosomal and mitochondrial loci are unlinked. We consider three sets of biological scenarios (unique combination of parameters), defined by the number of hybridizations occurring (one or unlimited) and by the geographic stability of the two habitats (fixed or shifting boundary), as detailed below. Note that the definition of these three sets of scenarios is independent of dispersal (which can be high or low in each scenario).

First set of scenarios: Sex-biases and selection on stable secondary contact

Both habitats are grids of 15 (on the X-axis) by 10 (on the Y-axis) demes of 20 individual each, and therefore, both taxa initially consist of $N_1 = N_2 = 3000$ individuals (we repeated the simulations with 50×10 demes without any relevant change in the results, except for a lower variability due to reduced drift). In this scenario, the secondary contact is stable (i.e., the spatial boundary between the two habitats is fixed). The three sex-biased processes suggested as possible causes of mito-nuclear discordant introgression can occur depending on the scenario considered. First, juvenile dispersal rate can differ between males and females. Second, hybrid males and females can differ in their juvenile survival probabilities. Third, intertaxa crosses can be sex-biased to favor the males of one taxon in which here are no limitations on the mating of females of taxon 2 with males of taxon 1 or with hybrid males, but the mating of females of taxon 1 with males of taxon 2 or with hybrid males is rejected with some probability. In addition, we consider scenarios including mitochondrial selection and scenarios with multilocus nuclear selection and partial linkage.

Second set of scenarios: Sex-biased dispersal during spatial invasion

As above, the starting population size of each taxon is large. However, in this set of scenarios, when the secondary contact starts, one of the habitats progressively expands at the expense of the other habitat until it occupies all the area. As a consequence, the taxon best adapted to the expanding habitat invades the whole area while the other taxon eventually goes extinct. In agreement with the verbal hypothesis proposed to explain mito-nuclear discordant introgression in the context of spatial invasions (Petit and Excoffier 2009), we modeled sex-biased dispersal of various strength and direction in this scenario.

Third set of scenarios: Single hybridization event in small population

In this set of scenarios, the two habitats contain a small number of demes so that the starting population size of each taxon is small $(N_1 = N_2 = 600, \text{ on a grid of } 10 \times 6 \text{ demes})$. Only one intertaxa mating involving pure individuals is allowed, while there is no restriction on matings involving hybrids or back-crosses. These

conditions seem favorable to a mito-nuclear discordance solely due to random genetic drift as more hybridizations would make it less likely that the introgressed mitochondria goes to fixation while all introgressed nuclear copies are lost or remain at low frequencies. No sex-biased processes are simulated in this set of scenarios.

SIMULATION ALGORITHM

Here we give a brief overview of the algorithm, for a detailed description see SI A.1. We first simulate the genetic polymorphism on the generation preceding secondary contact according to a standard coalescent process (Nordborg 2001). The secondary contact is then simulated using an individual-centered forward algorithm, under an isolation by distance model (Rousset 2004, p 24). In natural populations, dispersal often has a high kurtosis, that is, very long dispersal events are overrepresented (Kot et al. 1996). Here, this feature is represented using a geometric dispersal model, with the most important parameter being the individual probability of dispersal on one dimension (m). Since the grid has two dimensions, the total probability that a juvenile disperses is $1 - (1 - m)^2$ (ignoring boundary effects). Juveniles undergo viability selection that can be affected (depending on the scenarios) by mitochondrial lineage (independent of the habitat and of the nuclear background), hybrid status, or the match between local adaptation loci and habitat. Selection is controlled by the parameters ϕ_M for mitochondrial selection, $\phi_{h,Q}$ and $\phi_{h,Q}^{\gamma}$ for sexspecific selection against hybrids, and ϕ_n for local adaptation. One of the biological processes proposed to trigger mito-nuclear discordance involves asymmetrical interspecific mating. This could happen for instance if males of one taxon present exacerbated traits that are preferred by females of both taxa (Wirtz 1999). We simulated this process as nonrandom mating with respect to the local adaptation genotype (i.e., the species-determination genotype). The probability that a mating is successful is given by the matrix:

ç∖o	1 1	1 2	2 2
1 1	$\begin{pmatrix} 1 \end{pmatrix}$	ψ	ψ)
1 2	1	ψ	ψ
2 2	1	1	1)

where row and columns labels are local adaptation genotypes of females and males, respectively, and where $\psi \in]0; 1]$. When mating is not successful, the focal parent (female or male chosen with a probability of 0.5) then chooses a different partner, until mating is successful.

The algorithm was validated by comparing the identity probabilities of pairs of genes in the simulations, with simulations from the software IBDsim (Leblois et al. 2009) and with theoretical expectations (see SI A.3).

SIMULATION PARAMETERS

We simulated 100 replicates for scenarios with high dispersal rate, 299 replicates for scenarios with low dispersal rate and 1000 replicates for the scenario of a single hybridization event in a small population. We changed the number of replicates because: (1) The importance of genetic drift, and thus the probability of rare extreme discordance increases with decreasing gene flow (controlled by dispersal and constraints on intertaxa mating, see results); (2) Our simulations are computationally intensive, and we could afford 1000 replicates only for the third set of scenarios were we simulate fewer individuals and fewer generations. However, using only 100 replicates in all scenarios does not change our conclusions.

The secondary contact lasts for 6000 generations in the two sets of broad biological scenarios involving large population sizes and unlimited hybridizations (sets of scenarios one and two). Assuming a generation time of two years, this duration represents a secondary contact starting after the last glaciation and observed today, a situation thought to be common (Hewitt 1996; Melo-Ferreira et al. 2007; Renoult et al. 2009). For the "unique hybridization in small population" scenario, we simulate only 2000 generations after the beginning of the secondary contact, which is sufficient to fix an introgressed mitochondrial lineage most of the time (see Table SI C) given the small population size.

Gene flow between and within taxa is primarily controlled through dispersal (and the dispersal rate *m*), averaged over sexes. In the third set of scenarios, between taxa gene flow is also controlled by the restriction on the number of hybridization events. In initial simulations, a dispersal rate m = 0.1 produced mitochondrial captures in about 50% of the simulation replicates when none of the processes of interest was tested. We therefore used m = 0.1for scenarios with high introgression rates. A dispersal rate m = 0.001 produces low nuclear introgression: the mean proportion of autosomal loci with some introgression was less than 10% (see Results). Such a low autosomal introgression implies that a mitochondrial capture would produce MDMI, thus providing ideal conditions to test for factors that enhance mitochondrial introgression. We therefore used m = 0.001 for scenarios with low introgression rates. In all scenarios, the shape of the dispersal function (g parameter, see SIA.1) does not change, only its magnitude.

Sex-specific dispersal rates range between 0.001 and 0.199. They are combined so that the mean dispersal rate always equals 0.1 in the scenarios with high dispersal rate and 0.001 in the scenarios with low dispersal rate, and the ratio $\frac{m_{Q}}{m_{Q^3}}$ ranges between 0.005 and 199. Sex-specific relative hybrid survivals range between 0.001 and 0.599. They are combined so that the mean hybrid survival always equals 0.3 and they produce ratio $\frac{\Phi_{h,Q}}{\Phi_{h,Q^3}}$ ranging between 0.002 and 599. Sex-biased acceptance of intertaxa crosses (ψ) ranges between 0.01 and 1. A value of $\psi = 0.01$ means that matings involving a female of taxon 1, or an hybrid

female, and a male that is not of taxon 1, fail 99% of the time. A value of $\psi = 1$ means that intertaxa matings are not constrained. For details and other parameters values, see SI A.2.

DEFINITION OF INTROGRESSION MEASURES

To quantify introgression, we first sampled a fraction of individuals in a fraction of the simulated demes (in accordance with empirical studies, see SI A.4 for values). We then selected the taxon that was the most introgressed at the mitochondrial locus within each simulation replicate, and discarded the other taxon. Which taxon was used to quantify introgression thus varied among replicates. We denote M the proportion of introgressed mitochondrial copies in this taxon, f(M > x) the frequency of simulation replicates for which mitochondrial introgression in the sample was greater than x, and f(M = 1) the frequency of mitochondrial captures. We denote a_i the proportion of autosomal gene copies of introgressed origin at locus i, and $\mathbf{a} = (a_1, a_2, \dots a_{20})$. $p(\mathbf{a} > x)$, is the proportion of autosomal loci whose introgression frequency is higher than x, within a simulation replicate (and $p(\mathbf{a} = 1)$ is the proportion of autosomal loci that are captured). $p(\mathbf{a} > x)$ and $p_{\min}(\mathbf{a} > x)$ are respectively the average and the minimum of $p(\mathbf{a} > x)$ over simulation replicates of a given scenario. The mean proportion of autosomal gene copies of introgressed origin, averaged over all autosomal loci within a simulation replicate is written $\bar{\mathbf{a}}$.

Finally, the mito-nuclear introgression discordance is quantified as $M - \bar{\mathbf{a}}$. This difference equals 1 when mtDNA is captured and there is no autosomal introgression. We consider that there is MDMI when $M - \bar{\mathbf{a}} > 0.8$. This threshold was chosen because (i) in case of a mitochondrial capture, an autosomal introgression greater than 20% is likely to be easily detected; (ii) this threshold is about 0.1 above the maximum discordance observed in our simplest scenario, where none of the mechanisms proposed to cause MDMI are included (except for a narrow window of dispersal rate, see Results).

Empirical studies usually detect nuclear introgression through comparisons of introgressed versus nonintrogressed populations using exploratory approaches such as comparisons of F_{ST} , principal component analysis (Cavalli-Sforza 1966), modelbased assignment (Piry et al. 2004) or clustering methods (Pritchard et al. 2000). Here we bypassed this step by tracing the taxonomic origin of every gene copy and thus directly quantify introgression without error. In empirical studies, such a simple situation could be encountered if diagnostic genetic diversity had been identified in "pure" populations of the two taxa, outside the zone of secondary contact.

ANALYSIS OF MITOCHONDRIAL SELECTION

For simulations with mitochondrial selection, we assessed molecular evidence of past selection using Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) in Arlequin ver 3.5.1.3 (Excoffier et al. 2005). For every simulation replicate, we recorded the *P*-value from the test of the significance of the *D* statistic and of the F_S statistic, within the introgressed taxon, that the taxon having initially a disadvantaged mitochondrial lineage. Thus, we assessed the statistical power of the tests for different strength of mitochondrial selection. The statistics were computed within taxa, and tested against 1000 coalescent simulations generated under selective neutrality and population equilibrium.

Results

EFFECT OF DISPERSAL RATE IN STABLE SECONDARY CONTACT

We first explored the rates of autosomal and mitochondrial introgression as a function of dispersal rate to find dispersal rates favorable to testing the different hypotheses for the production of MDMI.

When dispersal rates are too high, autosomal introgression is large (Fig. 2B) and does not allow for MDMI, whatever the mitochondrial introgression (Fig. 2C). On the other hand, when dispersal rates are too low, mitochondrial introgression is low (Fig. 2A), and MDMI is not possible either (Fig. 2C). There is, however, a narrow window of dispersal rates where a few cases of MDMI occur: for m = 0.01, 7% of the replicates, with a maximal discordance of 0.92; for m = 0.02 and m = 0.03, 3% of the replicates, both, with a maximal mito-nuclear discordance $(M - \bar{a})$ of 0.87 and 0.81, respectively (Fig. 2C). We do not focus further on this window because MDMI happens here due to drift, which makes it difficult to isolate the effect of other processes.

For m = 0.1 the proportion of introgressed mitochondrial copies is high (M = 0.46), but MDMI never occurs because autosomal introgression is also high. In these conditions, a process that would decrease nuclear introgression could produce MDMI; provided this does not simultaneously decrease mitochondrial introgression. Therefore, in the first section, we use m = 0.1 to test processes that may produce MDMI.

Only for m = 0.001 is the proportion of introgressed autosomal copies (\bar{a}) always below 20% (Fig. 2B). Here this low autosomal introgression does not lead to MDMI because mitochondrial introgression is low (Fig. 2A). Nevertheless, a mitochondrial (quasi-)capture would produce MDMI —unless the process involved increases autosomal introgression as it increases mitochondrial introgression. Therefore, in the second section we keep m = 0.001 to test processes that may produce MDMI by favoring mitochondrial introgression.

HIGH DISPERSAL RATE

In this section, the dispersal rate is high (m = 0.1), leading to a high hybridization and introgression rates (Fig. 2). We look for

conditions minimizing nuclear introgression, while maintaining high mitochondrial introgression.

Stable secondary contact

MDMI does not occur when none of the hypotheses proposed in the literature are considered (i.e., no sex-bias in dispersal, hybrid fitness, or intertaxa mating, no mitochondrial selection, no autosomal linkage, and a large population) under a geographically stable secondary contact and with high dispersal rates. The discordance $(M - \bar{\mathbf{a}})$ is positive in most simulations replicates (indicating more mitochondrial than autosomal introgression), but is always less than 0.7 (Fig. 5A with $\frac{m_{\varphi}}{m_{Q'}} = 1$). Mitochondrial captures are more frequent (f(M = 1) = 0.56, SE=0.02) than captures of autosomal loci (on average 6% over loci and replicates). Autosomal introgression is however always substantial and sufficiently high to be easily detected. Indeed, about half of autosomal gene copies are of introgressed origin (mean $\bar{\mathbf{a}} = 0.48$, SE=0.03; minimum $\bar{\mathbf{a}} = 0.32$) and most markers have more than 10% of their copies of introgressed origin ($\overline{p(\mathbf{a} > 0.1)} = 0.85$, SE=0.07) (Fig. 4A with $\frac{m_{\varphi}}{m_{a}} = 1$). This baseline scenario never allows for substantial mitochondrial introgression without having a level of autosomal introgression that would be easy to detect in empirical studies.

Sex-biased dispersal. Female-biased dispersal only marginally increases mito-nuclear discordance $(M - \bar{\mathbf{a}})$ (Fig. 5A, $\frac{m_Q}{m_{q^2}} > 1$). Increasing female-biased dispersal favors mitochondrial introgression and mitochondrial capture (Fig. 3A, although the frequency of mitochondrial capture levels off above $\frac{m_{\varphi}}{m_{z}} = 3$) but autosomal introgression is not affected by sex-biased dispersal (Fig. 4A). There is little variation in autosomal introgression among simulation replicates, and the minimal values of autosomal introgression are close to the medians. For instance for $\frac{m_{Q}}{m_{Q^2}} = 199$, $\bar{\mathbf{a}}$ is within the range [0.33, 0.58] with a mean of 0.46. Therefore it would be implausible not to detect autosomal introgression when a mitochondrial capture occurs. Last, male-biased dispersal (Fig. 5A, $\frac{m_{\varphi}}{m_{\sigma^2}} < 1$) decreases mito-nuclear discordance down to negative values, so that there is more nuclear than mitochondrial introgression. Thus, although female-biased dispersal has an effect on the frequency of mitochondrial capture and introgression, it has no effect on any aspect of autosomal introgression, and cannot produce MDMI under these conditions.

Sex-biased hybrid survival. Sex-biased hybrid survival does not produce MDMI. Higher female hybrid survival does not increases mito-nuclear discordance, while higher male hybrid survival decreases it (Fig. 5C). The lack of effect of sex-biased hybrid survival can be understood as follows: increasing survival of hybrid females relative to hybrid males increases mitochondrial introgression and mitochondrial capture (Fig. 3C) but does



Figure 2. Mitochondrial and autosomal introgression rates as a function of dispersal rate (*m*). (A) The proportion of replicates of a scenario showing mitochondrial capture \pm SE (f(*M* = 1), in black) and the distribution of the proportion of introgressed mitochondrial copies (*M*, gray whisker boxes). (B) Nuclear introgression: the proportion of introgressed autosomal copies over all loci (ā, black circles and line) and the proportion of autosomal loci with at least 10% of introgressed copies (p(a > 0.1), gray squares and line). Both statistics are given with the means, 95% value intervals (thick error bars) and the 100% value intervals (thin error bars). (C) Mito-nuclear discordance: proportion of mitochondrial copies of introgressed origin minus proportion of autosomal copies of introgressed origin. The dashed horizontal line indicates a discordance of 80% (see definition of introgression measures for the choice of this threshold).

not decrease autosomal introgression (Fig. 4C), which remains substantial. Therefore, the increase in mitochondrial introgression due to higher survival of female hybrids cannot result in MDMI (i.e., a discordance above 80%). Last, when hybrid females survive less, fewer hybrid females reproduce, lowering mitochondrial gene flow and decreasing the level of mito-nuclear discordance.

Asymmetric crosses. Asymmetric crosses do not produce MDMI, but the mean mito-nuclear discordance increases with more asymmetric crosses (smaller ψ , see Table 2) (Fig. 5E). The asymmetry of the mating preferences makes mitochondrial introgression more common from taxon 2 into taxon 1, and this directionality increases with increasing ψ : for $\psi = 1$ the introgression goes from 2 to 1 50% of the time, while this frequency reaches 99% for $\psi = 0.01$. As with sex-biased dispersal and hybrid fitness, more asymmetric crosses do increase the extent of mitochondrial introgression (Fig. 3E), but decrease only slightly autosomal introgression (for $\psi = 1$ mean $\bar{\mathbf{a}} = 0.47$, minimum $\bar{\mathbf{a}} = 0.32$; for $\psi = 0.01$ mean $\bar{\mathbf{a}} = 0.45$; minimum $\bar{\mathbf{a}} = 0.25$; see also Fig. 4E in SI), and thus cannot produce a strong discordance. Results were identical when mating probabilities were determined by the multiplicative effects of 20 loci instead of a single one (see Fig. D.1 in SI).

Combination of the three sex-biased processes. Empirical studies invoking sex-biased fitness or behavioral traits to explain mito-nuclear discordance often suggest that the three processes tested above might contribute together to the observed patterns (e.g., Roca et al. 2005). We simulated these processes together to test for the possibility of interacting effects. We simulated all combinations of $\frac{m_{Q}}{m_{Q^2}} = \{3, 19, 199\}, \frac{\phi_{h,Q^2}}{\phi_{h,Q^2}} = \{2, 11, 599\}$ and $\psi = \{0.9, 0.5, 0.01\}$. For one simulation replicate in the most extreme scenario $(\frac{m_{Q}}{m_{Q^2}} = 199, \frac{\phi_{h,Q^2}}{\phi_{h,Q^2}} = 599$ and $\psi = 0.01$), discordance was just above our threshold for MDMI: $M - \bar{\mathbf{a}} = 0.81$ as a result of a mitochondrial capture along with $\bar{\mathbf{a}} = 0.19$. Nevertheless, most of the distribution of discordance fell well below the threshold (Fig. 6A).

Mitochondrial selection and nuclear selection with linkage. As expected, mitochondrial selection increases mitochondrial



Figure 3. Mitochondrial introgression and sex-biases, with high (left column) and low (right column) hybridization rates. All graphs show two measures of introgression: (i) the proportion of replicates of a scenario showing mitochondrial capture $\pm 2 \times SE$ (f(M = 1) in black); (ii) the distribution of the proportion of mitochondrial copies of introgressed origin (M, gray boxplots). (A-F) Mitochondrial introgression in a stable secondary contact, as a function of sex-biased dispersal, sex-biased hybrid survival, or sex-biased inter-taxa crosses. (G-H) Mitochondrial introgression following the spatial invasion of one taxon into the range of the other one, as a function of sex-bias in dispersal. Discordance is quantified in the invaded area only, from the resident into the invasive species (introgressed alleles are from the taxon that was swamped during the invasion). In (A,B,G,H) the x variable is the ratio of female dispersal rate over male dispersal rate (m_{\odot}/m_{\odot}) , and is drawn on a log-scale. In (C,D) the x variable is the ratio female hybrids survival on male hybrids survival ($\phi_{h, \mathcal{Q}}/\phi_{h, \mathcal{O}}$) and is drawn on a log-scale. In (E,F) The x variable is the probability that a female having at least one allele 1 at the speciation locus accepts as a mate a male with at least one allele 2 (ψ). "High dispersal rate" column corresponds to a dispersal rate (averaged over sexes, m) of 0.1, "low dispersal rate" column to m = 0.001.

introgression, but cannot lead to MDMI here, because autosomal introgression remains high (see SI B.1).

As expected, we obtained MDMI by simulating strong linkage between multiple local adaptation nuclear loci. For the parameter values we considered, the number of local adaptation nuclear loci and the strength of local adaptation both had little effect on mito-nuclear discordance. In contrast, linkage strongly decreased autosomal introgression and led to frequent MDMI. See SI B.2 for detailed results.

Single hybridization event in small population. In this section, we explore the effect of strong genetic drift (small population



Figure 4. Autosomal introgression and sex-biases, with high (left column) and low (right column) hybridization rates. All graphs show two measures of introgression: (i) the proportion of introgressed autosomal copies over all loci (\bar{a} , in black); (ii) the proportion of autosomal loci with at least 10% of introgressed copies (p(a > 0.1), in gray). Both measures are shown with their mean (circle), 95% range interval (thick line) and 100% range interval (thin line). The figure labels A–H correspond to the same scenarios as in Fig. 3. See Fig. 3 for details.

sizes $N_1 = N_2 = 300$ individuals) when hybridization is rare (a single hybridization event allowed, assuming strong reproductive isolation between the two pure taxa). Dispersal is independent of hybridization rates here, but we consider high dispersal rates (m = 0.1) to reach an equilibrium quickly.

With a single hybridization event in a small population, it is possible to obtain MDMI, even though it remains very rare (Fig. SI D.2). With a hybrid survival $\phi_h = 0.3$ we obtained a single case of MDMI out of 1000 simulations replicates, consisting in a complete mitochondrial capture without any autosomal introgression. On average this scenario leads to low introgression at both autosomal and mitochondrial loci, with around 1% of gene copies of introgressed origin. We obtained 29 replicates with mitochondrial captures, but 28 of them also had high autosomal introgression (Table SI C). Increasing the hybrid survival parameter ϕ_h to 0.9 increased the frequency of replicates with high mitochondrial introgression but also increased autosomal introgression (Table SI C). For instance, in the cases of mitochondrial capture, the autosomal introgression was high on average (Table SI C) and never below 0.55.



Figure 5. Mito-nuclear discordance and sex-biases, with high (left column) and low (right column) hybridization rates. (A–F) Discordance in a stable secondary contact, as a function of sex-biased dispersal, sex-biased hybrid survival, or sex-biased inter-taxa crosses. (G–H) Discordance following the spatial invasion of one taxon into the range of the other one, as a function of sex-bias in dispersal, and discordance is quantified in the invaded area only, from the resident into the invasive species (introgressed alleles are from the taxon that was swamped during the invasion). In all graphs, the *y*-axis represents mito-nuclear discordance, the proportion of mitochondrial copies of introgressed origin minus proportion of autosomal copies of introgressed origin, and the dashed horizontal line indicates a discordance of 80%. The figure labels A–H correspond to the same scenarios as in Fig. 3. See Fig. 3 for details.

Thus when hybrid fitness is low, mitochondrial captures can occasionally be associated with low autosomal introgression but are very rare, and when hybrid fitness is high, mitochondrial captures are likely, but they are never associated with low autosomal introgression.

Secondary contact followed by a spatial invasion

In the context of spatial invasion, sex-biased dispersal affects the relative rates of autosomal versus mitochondrial introgression (Petit and Excoffier 2009) and generates introgression from the resident into the invading taxon (Currat et al. 2008). In this set of scenarios, we quantify introgression from the shrinking taxon into the expanding taxon and model the same levels of sex-biased dispersal as in the previous section. A single habitat remains at the end of the secondary contact, but we consider that the former split is tractable (e.g., because the former boundary between the habitats corresponds to a geographic barrier) and measure introgression only on the invaded area.

In the absence of sex-biased dispersal, the discordance is negative on average, that is there is more autosomal than

Simulation variables	Definition
N_i	Population size in habitat <i>i</i> at any time, population size of taxon <i>i</i> during allopatry
X and Y	Dimensions of the deme grid
$m, m_{\mathcal{Q}} \text{ and } m_{\mathcal{Q}}$	Mean and sex-specific one dimensional dispersal rate per generation
ϕ_M	Multiplicative effect on relative juvenile survival of a maladapted mitochondria
ϕ_n	Multiplicative effect on relative juvenile survival of a maladapted homozygote autosomal locus
ϕ_h , $\phi_{h,Q}$ and $\phi_{h,Q}$	Mean and sex-specific multiplicative effect on relative juvenile survival of a heterozygote
	autosomal locus
ψ	Mating acceptance rate of females having at least one allele 1, for males with at least one allele 2
Introgression statistics	Definition
M	Proportion of introgressed mitochondrial copies in the taxon that is the most introgressed at this locus
f(M > x)	Frequency of simulation replicates with mitochondrial introgression in the sample greater than x
f(M = 1)	Frequency of simulation replicates with mitochondrial capture observed in the sample
a_i	Proportion of gene copies of introgressed origin at locus i
ā	Proportion of gene copies of introgressed origin, averaged over all autosomal loci
$p(\mathbf{a} > x)$	Proportion of autosomal loci whose introgression frequency is higher than x
$p(\mathbf{a} > x)$	Average of $p(\mathbf{a} > x)$ over simulation replicates of a given scenario
$p_{\min}(\mathbf{a} > x)$	Minimal $p(\mathbf{a} > x)$ observed over simulation replicates of a given scenario
p(a = 1)	Proportion of autosomal loci that are captured
$M - \bar{\mathbf{a}}$	Mito-nuclear discordance



Figure 6. Distribution of the mito-nuclear discordance for scenarios including sex-biased dispersal, sex-biased hybrid survival, and asymmetrical intertaxa crosses, with (A) high and (B) low dispersal rates. The discordance is quantified as the difference between the frequency of mitochondrial copies of introgressed origin minus the frequency of autosomal copies of introgressed origin. The dashed horizontal line highlights 80% of discordance. The y-axis represent the frequency of the classes of discordance and is drawn on a natural scale (A) or a logarithmic scale (B).

mitochondrial introgression, and the variance in discordance is very large (Fig. 5G with $m_{Q}/m_{C^{7}} = 1$). MDMI is never observed, whatever the ratio of sex-specific dispersal. Mitochondrial introgression goes up with more male-biased dispersal, as described in Petit and Excoffier (2009), and mitochondrial capture is almost certain for the most male-biased dispersal (85% of simulation replicates, see also Fig. 3G). Autosomal introgression does not decrease as mitochondrial introgression increases, however (Fig. 4G). For m_{φ}/m_{σ} = 199 the mean of $\bar{\mathbf{a}}$ is 0.46 (SE 0.03) and its minimum is 0.31; for $m_{\varphi}/m_{\sigma} = 0.005$, the mean is 0.45 (SE 0.03) and the minimum 0.32. Therefore, higher female dispersal has no effect on mito-nuclear discordance, while higher male dispersal decreases the variance in discordance and increases the mean discordance to around 0.55 (Fig. 5G).

LOW DISPERSAL RATE

So far we have explored scenarios with overall high introgression rates. While some parameters influence mitochondrial introgression, autosomal introgression is barely affected by them (except for nuclear selection) and MDMI is very rare. We now simulate low dispersal rates (m = 0.001), which leads to low introgression rates, and look for processes that could increase mitochondrial introgression while maintaining low autosomal introgression.

Sex-biases and asymmetric mate choice with stable secondary contact

Sex-biased dispersal, hybrid survival, or asymmetric mating patterns never produce MDMI with low dispersal rates in a stable secondary contact. Firstly, autosomal introgression does not change substantially with sex-biased dispersal (Fig. 4B), sex-biased hybrid survival (Fig. 4D), or asymmetrical crosses between taxa (Fig. 4F). Secondly, mitochondrial introgression is affected by these three processes, but they tend to decrease rather than increase discordance. More specifically, male-biased dispersal lowers mitochondrial introgression, and therefore discordance, while

ϕ_M	$\mathbf{f}(M=1)$	\overline{M}	ā	$\overline{M} - \overline{\overline{\mathbf{a}}}$	\bar{D}	$\mathrm{f}(p_D < 0.05)$	$ar{F_S}$	$\mathrm{f}(p_{F_S} < 0.05)$
1.000	0.000	0.009	0.005	0.004	-0.112	0.037	8.521	0
0.998	0.000	0.011	0.005	0.006	-0.086	0.036	8.592	0
0.995	0.000	0.011	0.005	0.005	-0.092	0.045	9.059	0
0.993	0.000	0.014	0.005	0.008	0.077	0.057	10.437	0
0.990	0.000	0.015	0.005	0.009	0.803	0.062	12.274	0
0.975	0.000	0.058	0.006	0.052	2.194	0.035	17.906	0
0.950	0.003	0.261	0.006	0.254	2.375	0.049	19.797	0
0.925	0.061	0.575	0.007	0.568	2.143	0.036	15.982	0
0.900	0.424	0.873	0.007	0.865	0.413	0.098	10.065	0
0.800	1.000	1.000	0.008	0.992	-0.167	0.000	2.454	0
0.700	1.000	1.000	0.007	0.993	-0.184	0.006	2.826	0

Table 3. Tests of neutrality of the captured mtDNA within the capturing taxon in the case of low dispersal rates.

 ϕ_M is the fitness disadvantage of the invaded mitochondria compared to the invading mitochondria of fitness 1. f(M = 1) is the frequency of mitochondrial captures, \overline{M} is the mean proportion of introgressed mitochondrial copies, \overline{a} is the mean proportion of introgressed copies over autosomal markers and $\overline{M} - \overline{a}$ is a measure of mito-nuclear discordance, all averaged over simulation replicates. \overline{D} is the mean Tajima's D and $\overline{F_s}$ the mean Fu's F_s observed over 299 simulation replicates. f($p_D < 0.05$) and f($p_{F_s} < 0.05$) give the statistical power of Tajima's D tests and of Fu's F_s tests, carried out in Arlequin V 3.5.1.3

female-biased dispersal only increases slightly mitochondrial introgression relative to the scenario with equal dispersal rates (Figs. 3B and 5B). Similarly, male-biased hybrid survival lowers mitochondrial introgression and discordance, while higher relative female hybrid survival only slightly increases mitochondrial introgression (Figs. 3D and 5D). Finally, there is no substantial effect of the direction of crosses between taxa on any aspect of introgression and discordance (Figs. 3F, 4F and 5F).

Moreover, there is a small positive correlation between M and \bar{a} (Pearson's product-moment correlation 0.081), which attenuates the discordance in cases of large mitochondrial introgressions. Nevertheless, the distribution of discordances with femalebiased processes (female higher dispersal, hybrid survival, and/or intertaxa mating rate) is right-shifted and reaches higher values than the distribution produced in the absence of any sex-bias (Fig. 6B). Discordances above 0.4 remain scarce (0.2% of the simulation replicates across all scenarios with sex-biases). Overall, the highest discordance observed is 0.74, despite the extreme values of sex-biased processes simulated simultaneously: in the most extreme scenario, females disperse 199 times more than males, survive 599 times more and refuse to mate with 99.9% of the males with the wrong genotype.

Mitochondrial selection

MDMI is easily observed when sufficient mitochondrial selection is simulated. Indeed, mitochondrial capture is then always observed while autosomal introgression does not change with the strength of mitochondrial selection when relative fitness of the less fit lineage varies between $\phi_M = 0.7$ and $\phi_M = 1$. Mitochondrial capture, and therefore MDMI, is reached in all simulation replicates for a fitness of the disadvantaged lineage $\phi_M \le 0.8$ (Table 3). Nevertheless, the two common tests of neutral evolution applied on the introgressing mtDNA have a low statistical power (Table 3). Fu's F_S test never rejects the null-hypothesis of neutral evolution. Tajima's D test rejects the null-hypothesis of neutral evolution with a frequency close to the expected false-positive rate of 5% under neutrality. The statistical power, how-ever, is almost zero when mitochondrial capture occurs, that is, when mitochondrial selection is the strongest (Table 3). Statistical power is highest, but still below 10%, with moderate selection and incomplete mitochondrial capture.

Secondary contact followed by a spatial invasion

There is no MDMI observed with the scenarios of spatial invasion and mito-nuclear discordance is close to zero. Moreover, sex-biased dispersal has little effect on the mean discordance, although male-biased dispersal reduces the variance in discordance (Fig. 5H).

To obtain a complete invasion (i.e., the disappearance of the local adaptation allele initially present in the invaded area) with a low dispersal rate (m = 0.001), the parameter controlling the survival of maladapted individuals (ϕ_n) was set to 0.1 instead of 0.9 as in previous scenarios.

Mitochondrial introgression is always high and is strongly favored by male-biased dispersal (Fig. 3H). Mitochondrial capture is frequent (> 50% of simulation replicates) for all values of sexbiased dispersal, and increases with male-biased dispersal to the point where mitochondrial capture is certain $(m_{\varphi}/m_{\sigma} = 0.005)$. On the other hand, autosomal introgression, is not sensitive to sex-biased dispersal, but is always very high (Fig. 4H). Therefore, mean mito-nuclear discordance is close to zero for all values of sex-biased dispersal, with very little variation when males disperse much more than females, and with negative discordance (i.e., more autosomal than mitochondrial introgression)

Dispersal	Baseline	Invasion	Sex-biases	Mitochondrial selection	Nuclear selection	Single hybridization
High	No	_	_	_	_	_
	-	Very rare ¹	-	-	-	-
	_	-	No	-	-	-
	-	-	-	No	-	-
	_	-	_	-	Yes	-
	-	-	-	-	-	Very rare ²
Intermediate	Rare ³	-	_	-	-	-
Low	No	-	-	-	-	-
	-	No	-	-	-	-
	-	-	No	-	-	-
	-	-	-	Yes	-	-

Table 4. Overview of the results: What produces MDMI.

Each line represents a broad class of biological scenarios, defined by dispersal rate (*m*), the presence of sex-biases (in dispersal, hybrid survival, or mating asymmetry), the occurrence of a biological invasion, the presence of strong selective nuclear barriers to introgression, and the presence of selective variation among the two mitochondrial lineages. The presence of MDMI for each scenario we tested is indicated in the corresponding cell

¹The discordance was just above our threshold for MDMI in one replicate of the scenario with the most extreme sex-biases.

²MDMI was observed in one simulation replicate out of 2000.

³A few cases of MDMI were observed for a range of intermediate dispersal values (*m* within [0.01; 0.03]).

in some simulation replicates when dispersal is female-biased (Fig. 5H).

pirical studies and the hypotheses of our model can complicate comparisons with real situations.

Discussion

We have explored what conditions can generate discordance in introgression level between nuclear and mitochondrial markers. Our results show that it is essential to distinguish between "massive" discordance, here defined as (near) fixation of mtDNA in foreign nuclear background without easily detectable nuclear introgression (results summarized in Table 4), and biologically significant but smaller differences in introgression level between both types of markers. The most common explanation for mito-nuclear discordance in introgression (massive or not) is sex-biased demographic traits (dispersal, hybrid fitness, or mate choice, see Funk and Omland 2003; Toews and Brelsford 2012). Our simulations show a more complex pattern. We find that massive discordance without selection is extremely rare and its occurrence is not primarily influenced by sex-biases in demographic processes. On the other hand, lower but biologically significant mito-nuclear discordance can be produced by mt-neutral processes such as sex-biases in demographic traits.

As expected, positive selection on mitochondria easily fixed the introgressing mitochondria and generates massive discordance, even if selection cannot be detected by classical tests of neutrality. Perhaps less intuitively, the spreading of selective genes along the chromosomes can effectively block nuclear admixture without preventing mitochondrial introgression, resulting in massive discordance for a wide range of plausible parameters. We will discuss these points below before examining how the limits of em-

SEX-BIASED DEMOGRAPHIC PROCESSES DO NOT PRODUCE MASSIVE DISCORDANCE

We have shown here that mt-neutral processes do not normally produce massive discordance (MDMI, discordance above 0.8). Sex-biased demographic processes (dispersal, hybrid fitness, or asymmetric intertaxa crosses) is the most common explanation for mito-nuclear discordance (Toews and Brelsford 2012). However, this explanation does not explicitly provide a mechanism for low nuclear introgression conditional to high mitochondrial introgression. Whatever the sex-biases, mitochondrial capture is unlikely without large hybridization rates, which also promote nuclear introgression. For instance, verbal explanations that rely on higher female dispersal to explain discordance fail to acknowledge that female carry just as many copies of nuclear genes as males, and thus disperse nuclear genes at the same time they disperse mitochondria.

Chan and Levin (2005) have already showed analytically that higher mitochondrial introgression is expected under a wide range of neutral conditions but found no conditions for which the discordance was massive. Our simulations support this view: Although discordance can be substantial, MDMI does not generally occur and autosomal introgression is always high and easily detectable. Even extreme sex-biased dispersal, hybrid survival, asymmetric crosses, or the combination of the three processes barely reduce nuclear introgression whatever the global hybridization rate or the situation (stable contact or invasion). In two simulation contexts, drift can produce MDMI with low frequency. Firstly, single-hybridization events in small populations produced MDMI in one out of 2000 replicates (in this case consisting in a complete mitochondrial capture without any autosomal introgression). Secondly, when hybridization is very rare, variance in discordance is high and discordance sometimes reaches value above 0.8 with average nuclear introgression below 0.2 (Fig. 2). Drift does not provide a general explanation for massive discordance as it produces MDMI very rarely, in specific contexts only.

DRIFT GENERATES HIGHLY VARIABLE, SOMETIMES SUBSTANTIAL LEVEL OF DISCORDANCE THAT IS INFLUENCED BY SEX-BIASED DEMOGRAPHIC PROCESSES

In most of the scenarios, there is a biologically significant mitonuclear discordance, even when demographic processes are not sex-biased. The fact that this discordance is often positive (more mitochondrial than nuclear introgression) is an artifact of our decision to record the discordance in the taxon with more mitochondrial introgression. Widespread mito-nuclear discordance is thus not necessarily a consequence of sex-biased dispersal, sex-biased hybrid fitness or asymmetric mate choice but is readily generated by drift, especially when dispersal is low. While MDMI never occurs under such conditions, some strong discordances in favor of mtDNA are observed at very low frequency. For instance, in the baseline scenario, mitochondrial introgression can reach up to 38% of gene copies, without a single introgressed autosomal gene copy.

Sex-biases in demographic processes are unable to generate massive discordance alone, but they influence the sign and extent of discordance (Fig. 5A–F). Sex-biased dispersal has the most dramatic effect (Fig. 5). In stable secondary contact (Fig. 5A, B), higher male dispersal decreases discordance relative to unbiased dispersal, while higher female dispersal has a very limited effect relative to unbiased dispersal. In cases of spatial invasion, the amount of discordance in the expanding species depends again on the level of dispersal: it is substantial with high dispersal but usually very small in case of low dispersal (Fig. 5G, H). When dispersal is high, male-biased dispersal dramatically increases discordance, however, while higher female dispersal has little effect compared with the balanced dispersal case (Fig. 5G). When dispersal is low (Fig. 5H), the effect of sex-biased dispersal is broadly similar but smaller.

Petit and Excoffier (2009) reviewed the published patterns of introgression for 38 taxa and found that species with femalebiased dispersal all had higher rate of nuclear introgression than of mitochondrial introgression, while species with male-biased dispersal tended to show the opposite pattern. This was thought to provide empirical support for spatial invasions being a general explanation for mito-nuclear discordances. However, a later more comprehensive review failed to confirm this pattern, with several examples of discordant introgression going in the direction opposite to what sex-biased dispersal predicted (Toews and Brelsford 2012). Our results help to understand the complexity of the relationships between patterns of discordance and biological traits of the species: negative discordance can be observed in stable secondary contacts when dispersal is male-biased or in moving contact zones (spatial invasion) in all cases but extreme malebiased dispersal. Moreover, long-distance dispersal can prevent introgression in the case of spatial invasion and sex differences in the shape of dispersal may lead to various pattern of discordance after a spatial invasion (Amorim et al. 2016). Last, we analyzed introgression at the global scale of an invaded area, but discordance might be stronger locally, especially on an invasion wave front; further work is needed to examine how discordance varies locally along invasion wave fronts.

ADAPTIVE MITOCHONDRIAL INTROGRESSION PRODUCES MASSIVE DISCORDANCE BUT LEAVES NO SIGNAL OF PAST SELECTION

As expected, a selective advantage of the mitochondrial lineage of one taxon over the mitochondrial lineages of the other taxon easily leads to mitochondrial captures in our simulations, and these captures result in MDMI when overall introgression rates are low. Perhaps more surprisingly, the Tajima's D and Fu's F_S tests were unable to detect mitochondrial adaptive introgression in our simulations, even with strong mitochondrial selection. These tests rely on the reduction of haplotypic diversity following a strong selective sweep. However, the adaptive introgression that we modeled is a soft sweep due to the introgression of multiple haplotypes of one taxon, selected over all the mitochondrial haplotypes of the other taxon. A large proportion of the genetic variation of the introgressing lineage is therefore preserved in the introgressed taxon, and there is not a strong molecular signal of the selective event (Pennings and Hermisson 2006).

Many classical studies of discordant mtDNA introgression report introgression of multiple haplotypes, a pattern consistent with the soft sweep hypothesis (e.g., Roca et al. 2005; Berthier et al. 2006; Melo-Ferreira et al. 2007; Renoult et al. 2009; Boratynski et al. 2011; Bastos-Silveira et al. 2012; Wielstra and Arntzen 2012; Zieliński et al. 2013; Melo-Ferreira et al. 2014). It is well known already that these classical neutrality tests have low statistical power in general (Wall 1999; Zhai et al. 2009), but here we show in addition that they will probably be unable to detect most cases of past mitochondrial adaptive introgression in the context of secondary contact. Thereby it does not come as a surprise that some studies applying these two tests in this context could not reject the null hypothesis of no selection (Melo-Ferreira et al. 2007; Good et al. 2008; Pons et al. 2013, but see Boratynski et al. 2011). Neutrality tests based on the comparison of several species (Hudson-Kreitman-Aguadé), could be more powerful (Zhai et al. 2009), although they require additional data.

A major assumption in our simulations is that the invading mitochondrial lineage is advantageous irrespective of its autosomal background. However most of the proteins acting in mitochondria are coded in the nucleus, and cyto-nuclear coevolution is likely to make some combinations more functional than others (Doi et al. 1999; Rand et al. 2004; Dowling et al. 2008). Such mito-nuclear interactions would probably make MDMI less likely since mitochondrial introgression would have to come along with introgression at the coadapted loci, thus decreasing the likelihood of mitochondrial capture and increasing the proportion of nuclear introgression conditional to mitochondrial capture. This also suggests that cases of mitochondrial introgression can provide a direct test of mito-nuclear coevolution: higher introgression of nuclear loci that interact with mitochondrial genes compared with random loci on populations showing mitochondrial introgression would support the coevolution hypothesis. A recent publication found some support for mito-nuclear interactions in the context of MDMI, although fitness could not be directly measured (Lee-Yaw et al. 2014).

SELECTION AGAINST INTROGRESSION IN THE NUCLEAR BACKGROUND CAN GENERATE MASSIVE DISCORDANCE

Funk and Omland (2003) suggested that mitochondrial alleles will be expected to introgress more on average than nuclear loci if the latter are less linked to selected loci. Our result support this idea: linkage between markers and selected loci increases discordance and can even produce MDMI when global dispersal rates are high and selection on the nuclear loci is strong. To prevent introgression of neutral nuclear markers at the genome scale, selection must be strong enough and spread over a sufficiently large number of loci, so that all markers are closely linked to some selected loci (Barton 1986; Barton and Bengtsson 1986). In our simulations, the strength of selection for local adaptation (ϕ_n) does not have an effect on nuclear introgression for the simulated values ($\phi_n = 0.1$ or 0.9). The number of loci under selection does not matter either (Fig. SI B.1 A). The important parameter is recombination: each marker needs to be closely linked to a locus under selection.

Selection against nuclear background can provide a valid explanation for empirical cases of massive mito-nuclear discordance when reproductive isolation can be reasonably expected to be coded by multiple loci spread regularly all over the genome. Coyne and Orr (2004) review on the number of loci involved in reproductive isolation (Table 8.2) suggests speciation genes are typically not very numerous but they only list genes involved in intrinsic postzygotic isolation identified via direct measures of hybrid fitness. The true number of loci under selection against introgression can be much higher if many loci have low effect (and would be hard to identify by their direct effect on fitness) and/or many loci are involved in extrinsic (ecological) or prezygotic isolation. Theoretical work suggests speciation can evolve indeed through the cumulative effects of numerous loci of small individual effects (Flaxman et al. 2014). Whether the hypothesis of nuclear background selection spread over the genome is realistic or not in many natural systems will require further data, as the evidence currently at hand is not sufficient to evaluate it.

MODEL ASSUMPTIONS AND LINKS TO EMPIRICAL STUDIES

In our simulations, the detection of nuclear introgression is perfect because we tracked the origin of every genetic lineages. In empirical studies, quantifying introgression is much less straightforward. Two main situations arise. In the most favorable case, pure populations of the species investigated have been sampled and a number of diagnostic loci have been identified and used for genotyping of introgressed populations. In this case, the measure of introgression is the proportion of introgressed alleles and is directly comparable to our results provided the number of diagnostic loci is high enough (at least 10, see the range of value for $p(\mathbf{a} > 0.1)$ Fig. 4). In many cases however introgression is estimated with nondiagnostic loci such as microsatellites, often with the use of clustering methods. Several articles (reviewed in Putman and Carbone 2014) have pointed out that the number of loci required for separating pure individuals from recent hybrids (F1, F2, backcrosses) is much higher than what is typically used in empirical studies (Fitzpatrick 2012; Vähä and Primmer 2006) and requires carefully designed sampling schemes including pure individuals (Vaughan et al. 2009). It is thus uncertain that the low level of nuclear introgression that goes with mitochondrial introgression in some of our simulations (\bar{a} as low as 0.19–0.25) would be easily identified in all empirical studies where mitonuclear discordance have been reported.

HOW COMMON IS ADAPTIVE MITOCHONDRIAL INTROGRESSION?

The data at hand do not allow to answer this question, but we are now in a better position to identify candidate empirical situations where adaptive mitochondrial introgression is the most likely explanation. Ideal candidates are situations where the introgressed haplotypes reach a frequency over 0.8 with nuclear introgression below 0.2, while case of (near)complete mtDNA replacement without nuclear introgression are unlikely to be caused by anything but adaptive mtDNA introgression. Unfortunately, few empirical studies report cases of mtDNA introgression together with robust measures of nuclear introgression, and with an assessment of mitochondrial selection. We do not pretend to give a comprehensive overview of the literature, but wish to point out a few studies suggestive of adaptive mitochondrial introgression when evaluated against our results. Boratynski et al. (2011) report complete fixation of *Microtus rutilus* haplotypes, related to variation in metabolic rates, in northern Scandinavian populations of *M. glareolus* without any introgressed allele of *M. rutilus* at six nuclear loci. If the lack of nuclear introgression was confirmed with a few additional markers, it would constitute a very strong case for adaptive introgression. Bachtrog et al. (2006) report a strong mito-nuclear discordance in the *Drosophila yakuba* species group and the molecular details of mito-nuclear cointrogression suggests another case of adaptive mtDNA introgression, albeit one involving coadaptation with nuclear genes (Beck et al. 2015).

CONCLUSION

We have shown that genetic drift generate more mito-nuclear discordance than usually realized but that sex biases in demographic processes such as dispersal or hybrid fitness have a limited impact on discordance. Furthermore, the effect of the biological processes at stake (such as sex-biased dispersal) is more complex than suggested in the existing literature, as different combinations of species traits and historical situations can produce similar patterns of discordance. As a consequence, many cases of mitonuclear discordance reported in the empirical literature might be explained by drift-generated variance in introgression rather than by the sex biases that are often mentioned to explain them. Even if sex-biased demographic processes are involved it will often be difficult to infer their role without robust independent information on the biology of the investigated species.

We have also shown that massive discordance (MDMI) is far less easy to obtain without selection than previously suggested. Identifying massive discordance is not always straightforward, as many empirical cases of discordance might not have the power to pick up the moderate to low level of nuclear introgression that need to be excluded to identify massive discordance. The development of genome-scale sequencing will enable a more accurate characterization of genome-wide introgression and allow to better tease apart the various neutral and selective processes shaping hybridization patterns on real data (Ellegren et al. 2012; Poelstra et al. 2014; Hellenthal et al. 2014). Nevertheless, current inference methods fail to account jointly for biological processes that are crucial in shaping genome-wide patterns of differentiation and introgression such as time-variation in dispersal, genomewide heterogeneity in selection and recombination rates, and geographical population structure. Only with the development of more elaborate frameworks will an efficient use of genomic data be possible (Payseur and Rieseberg 2016). Until then, simulation studies, such as this one, provide a powerful way to test the credibility of specific hypotheses.

AUTHORS' CONTRIBUTION

PAC designed the study. TB, RL and FR wrote the simulation code with input from PAC. TB analyzed the simulations and produced statistics and graphs. All authors contributed to writing the manuscript.

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DATA ARCHIVING

Code available from the Dryad Digital Repository https://doi.org/ 10.5061/dryad.44sv0 (Bonnet et al. 2017), and from the GitHub repository https://github.com/timotheenivalis/CodeAllForward.git.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure B1: Mito-nuclear discordance as a function of nuclear genetic architecture.

- Figure D1: Asymmetry of crosses between taxa and the discordance of mitochondrial and autosomal introgression, with 20 loci for mate choice.
- Figure D2: Distribution of the mito-nuclear discordance with a single hybridization event in a small population.

Table C1: Mitochondrial and autosomal introgression from simulations of a single hybridization in a small population.