Published in "Molecular Biology and Evolution
doi: 10.1093/molbev/mst140, 2013"
which should be cited to refer to this work.

Title: Inferring the geographic mode of speciation by contrasting autosomal and sex-linked genetic diversity

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Abstract

When geographic isolation drives speciation, concurrent termination of gene flow among genomic regions will occur immediately after the formation of the barrier between diverging populations. Alternatively, if speciation is driven by ecologically divergent selection, gene flow of selectively neutral genomic regions may go on between diverging populations until the completion of reproductive isolation. It may also lead to an unsynchronized termination of gene flow between genomic regions with different roles in the speciation process. Here we developed a novel Approximate Bayesian Computation pipeline to infer the geographic mode of speciation by testing for a lack of postdivergence gene flow and a concurrent termination of gene flow in autosomal and sex-linked markers jointly. We applied this approach to infer the geographic mode of speciation for two allopatric highland rosefinches, the vinaceous rosefinch *Carpodacus vinaceus* and the Taiwan rosefinch *C. formosanus* from DNA polymorphisms of both autosomal and Z-linked loci. Our results suggest that the two rosefinch species diverged allopatrically approximately 0.5 million years ago. Our approach allowed us further to infer that female effective population sizes are about five times larger than those of males, an estimate potentially useful when comparing the intensity of sexual selection across species.

Introduction

Geographic isolation that interrupts genetic exchange between populations has long been considered essential for species genesis (Mayr 1942, 1963; Coyne and Orr 2004). However, accumulating evidence suggests that speciation may often be driven by mechanisms other than geographic isolation such as ecologically divergent selection (reviewed by Nosil 2012). During speciation driven by ecologically divergent selection (i.e. ecological speciation), gene flow may continue for selectively neutral regions until the completion of reproductive isolation between populations, but not in genomic regions associated with divergent selection (Wu 2001). Therefore, a complete interruption of gene flow is unnecessary in the incipient stage of speciation. Consequently, the divergence dates of different genomic regions are predicted to be unsynchronized if ecological speciation occurred in parapatry or sympatry.

For organisms with heterozygous sex chromosome systems, autosomes and sex chromosomes could play unequal roles in speciation (Qvarnström and Bailey 2009). Dominance theory (Turelli and Orr 1995) predicts a stronger selection on sex chromosomes than on autosomes (Charlesworth et al. 1987; Ellegren 2009a; Hammer et al. 2010) and explains a higher genomic incompatibility and a lower fitness of hybrids in the hemizygous sex (i.e. males in the XY system and females in the ZW system; Haldane's rule, Haldane 1922). In the ZW system, theoretical and empirical works suggest that the Z chromosome plays a substantial role in driving prezygotic isolation by accumulating more mutations associated with sexually selected traits of males and mate preference traits of females (Kirkpatrick and Hall 2004; Ellegren 2009b; Qvarnström and Bailey 2009; Hogner et al. 2012). These trait differences can promote assortative mating for individuals from ecologically divergent populations

and facilitate speciation with gene flow (Dieckmann and Doebeli 1999; Doebeli and Dieckmann 2000; Schluter 2009). Therefore, the Z chromosome is thought to play a disproportionate role in speciation by promoting both pre- and post-zygotic reproductive isolation (Jablonka and Lamb 1991; Qvarnström and Bailey 2009). In birds, restricted postdivergence gene flow in Z-linked loci has been observed in speciation of parapatrically distributed flycatchers (Sætre et al. 2003), nightingales (Storchová et al. 2010) and passerina buntings (Carling et al. 2010). Furthermore, a much higher level of species divergence and a lower ratio of shared polymorphisms in the Z chromosome was reported in comparisons of the genomes of pairs of parapatric *Ficedula* flycatchers (Ellegren et al. 2012; Hogner et al. 2012). Thus, termination of gene flow for the sex chromosome might predate that for autosomes in the process of speciation.

Recent developments of analytical tools such as the isolation with migration model (IM, Nielson and Wakeley 2001; Becquet and Przeworski 2007; Hey 2010) and model selection procedures such as Approximate Bayesian Computation (ABC, Tavaré et al. 1997; Beaumont et al. 2002; Leuenberger and Wegmann 2010) have revolutionized our understanding of the geographic mode of speciation. With the aid of these approaches, accumulating evidences indicate that strict allopatric speciation advocated by Mayr (1942) might not be as common as we previously considered; instead, postdivergence gene flow is commonly found between species after their split (Nosil 2008). However, most of the newly developed tools only consider the lack of postdivergence gene flow when testing the geographic mode of speciation. Concurrent termination of gene flow among different genomic regions, is usually not incorparated in methods commonly used to test geographic mode of speciation.

Here, we report an ABC pipeline to test the geographic mode of speciation by inferring postdivergence gene flow and concurrent termination of gene flow from DNA polymorphisms of autosomal and sex-linked markers jointly. This approach is based on two competing models, a model of strict allopatry, with no gene flow since the two focal species started to diverge and a modified isolation with migration model allowing for postdivergence gene flow until complete isolation was established. The reasoning behind using these models is that if speciation was not strictly allopatric, i.e. in the absence of an initial geographic isolation, the isolation with migration model would be favored, and, if speciation was driven ecologically, an earlier interruption in gene flow is expected at sex-linked than at autosomal loci during the divergence process.

We illustrate the power and usefulness of our pipeline through an application to polymorphism data of both autosomal and Z-linked loci of the vinaceous rosefinch (*Carpodacus vinaceus*) and the Taiwan rosefinch (*C. formosanus*) (Wu et al. 2011). *C. vinaceus* is present in the Himalayas and the eastern edge of the Tibetan Plateau; and *C. formosanus* is restricted to Taiwan's montane regions (Hachisuka and Udagawa 1951; MacKinnon and Phillipps 2000). Both rosefinches are found at an elevation range of approximately 1800 to 3500 m (Hachisuka and Udagawa 1951; MacKinnon and Phillipps 2000; Robson 2000). As other *Carpodacus* finches, both *C. vinaceus* and *C. formosanus* are sexually dichromatic: males are almost entirely dark crimson, and females are deep tan-brown, suggesting the potential for strong selection on mate choice and reproductive success among males. However, in our analysis, a model of strict allopatry is highly favored for this species pair.

Results

ABC pipeline to infer geographic modes of speciation

Speciation models: We considered two competing speciation models (fig. 1): A) strict allopatry in which *C. vinaceus* and *C. formosanus* diverged instantaneously T_{div} generations ago and B) non-allopatry, or isolation with migration, in which the populations were allowed to exchange genetic material after divergence until complete isolation was established (referred to as the IM model below). In both models, the effective sizes of the two extant species were allowed to change at the time of split from the ancestral diploid size N_{anc} to long term effective sizes of *C. vinaceus* N_{vin} and *C. formosensis* N_{form}.

To take the difference in effective size between the autosomal and Z-linked markers into account properly, we assumed that the ratio of male and female effective population sizes $N_{m\_rel\_f} = N^m / N^f$ was constant through time and calculated the effective sizes as $N^{auto} = N^f \times (1 + N_{m\_rel\_f})$ and $N^Z = N^f \times (0.5 + N_{m\_rel\_f})$ for the autosomal and Z-linked markers, respectively. Under the assumption that both sexes are equal in number and females have similar levels of reproductive success, the effective sex ratio $N_{m\_rel\_f}$ is thus stronlgy associated with the variance of reproductive success (i.e. intensity of sexual selection) among males (e.g. Wade 1979). Therefore, this parameterization not only reduces the number of parameters in the model, but also provides extra information regarding to the relative intensity of male sexual selection of the diverging populations.

In the IM model, complete isolation was allowed to be established at different time points T_{iso}^{auto} for the autosomal and T_{iso}^{Z} for the Z-linked markers.

However, we assumed that genes were exchanged symmetrically at a rate of *2Nm* per generation at autosomal loci. The forward-in-time per-gene emigration rates were then computed as $m_{vin \rightarrow form} = 2N_{form}\, m / 2N_{vin}$ and $m_{form \rightarrow vin} = 2N_{vin} m / 2N_{form}$, respectively, and the same rates were used for both autosomal as well as Z-linked loci.

We chose to use the prior distributions and data set relevant for our application to the speciation history of rosefinches when evaluating the power of our approach, as the wide prior ranges encompass parameter combinations suitable for many bird or mammal species (table 1). For testing purposes, we thus generated many pseudo-observed data sets (PODS) of polymorphism data of 24 autosomal and 10 Z-linked loci with parameter values drawn randomly from these prior distributions.

Validation of parameter estimates: To validate parameter estimates of our pipeline, we inferred the marginal posterior distributions for all parameters independently for 5×10^4 PODS and recorded the smallest high posterior density interval (HPD) within which the true parameter values fall (supplementary fig. S1, Wegmann et al. 2009). Our validation analyses showed the marginal posterior distributions to be well calibrated for most parameters, indicated by a nearly uniform distribution of the recorded HPDs. However, as is expected for any approximate method, we found the posterior distributions to be slightly too broad (i.e., the true values falling too often into small HPD bins) for some parameters known to be notoriously difficult to estimate, in particular the migration rate and the two isolation times T_{iso_auto} and T_{iso_Z} of the IM model (e.g. Wegmann and Excoffier 2010).

Power to distinguish between models: We evaluated the power to distinguish between the two competing models in the rosefinches setting based on a large set of PODS. Specifically, we simulated 5×10^4 PODS for each model and performed model

choice on each of them individually. We found that our approach correctly identified the strictly allopatric and the IM model in 77.6% and 86.9% of the cases, respectively. Importantly, our pipeline provides an estimate of uncertainty (the posterior probability) along with the most likely model and we found that the average posterior probability of the true model was 0.28 when the wrong model was chosen. This suggests that the wrong model was rarely chosen with high confidence.

However, since model choice with ABC can easily be biased due to insufficient summary statistics (Robert et al. 2011), it is important to test if the estimated posterior probabilities are well calibrated. We thus compared the estimated posterior probability of the strict allopatry model against the empirical posterior probability, estimated as the proportion of data sets generated under the strict allopatry model falling in a given bin of ABC posterior probabilities (fig. 2). Although we found a slight bias towards a model with post divergence gene flow for intermediate posterior probabilities, our model choice inference appeared relatively unbiased in more decisive cases.

Power to detect differences in isolation times: To estimate the power and bias of our ABC approach to detect a difference in isolation times between autosomal and Z-linked loci of the IM model, we generated 1×10^4 PODS in which we sampled isolation times from their respective prior distributions; but fixed all other parameters to the values at the mode of the marginal posterior distribution obtained for the rosefinch data set under the IM model (see below). We then inferred the joint posterior distribution of T_{iso_auto} and $T_{iso\_Z,}$ and recorded the total posterior probability supporting $T_{iso\_auto} < T_{iso\_Z}$ (fig. 3). Since we found that the posterior distributions of both T_{iso_auto} and T_{iso_Z} were generally too broad (too conservative), the posterior

probability supporting $T_{iso\_auto} < T_{iso\_Z}$ is likely too conservative as well. Despite this and the very low migration rate inferred for our application under this model ($2Nm \approx 0.19$, see below), our analysis suggests that, at least for the parameter values relevant for this application, our ABC approach has considerable power to detect differences in the isolation times as soon as the two species became isolated > 100 generations ago.

Application to two highland rosefinches

We applied our ABC pipeline to sequence data from 24 autosomal and 10 Z-linked loci obtained for 25 and 29 individuals of *Carpodacus vinaceus* and *C. formosanus* respectively (supplementary fig. S2).

Genetic polymorphism and divergence: *C. vinaceus* showed higher nucleotide and haplotype diversity than *C. formosanus* in all sampled genes ($F_{5, 64}$=13.59, P<0.001, supplementary fig. S3, supplementary tables S1 and S2). We found lower diversity in the Z-linked loci than autosomal ones ($F_{5, 64}$=2.44, P=0.04, supplementary fig. S3, supplementary table S3) and more pronounced genetic differentiation at the Z-linked (mean Fst= 0.63) than the autosomal loci (mean Fst=0.44, $F_{1,31}$=4.90, P=0.04, supplementary fig. S3 and supplementary table S4). We also found more polymorphic sites shared between the two species at the autosomal loci (11 of 20) than the Z-linked loci (1 of 8, Fisher's exact test, P < 0.001, supplementary table S4). Furthermore, we discovered that molecular diversity ($F_{1, 64}$=0.01-2.04, P=0.16-0.93, supplementary fig. S3, supplementary tables S1 and S2) and genetic differentiation ($F_{1, 31}$=0.96, P=0.34, supplementary table S3) were not significantly different between exons and introns for either the autosomal or the Z-linked loci.

Neutrality tests: The multilocus HKA test suggested no departure from the neutral

expectation for either the autosomal (χ^2=29.76, d.f.=48, P=0.98) or the Z-linked loci (χ^2=9.58, d.f.=18, P=0.95). The tests of Fu and Li's *D* and Tajima's *D* showed no significant departure from neutrality for any loci except the exonic locus LOC100227384 (supplementary table S5). We therefore removed this locus from the subsequent analysis.

Evidence for allopatric speciation: The ABC analyses showed that the strict allopatry model was favored with a Bayes factor of 5.89 (a posterior probability of 0.86) compared with the IM model. Since we detected a slight bias in the posterior probabilities, we used the PODS to correct our posterior probabilities empirically (fig. 2). We still found substantial support for the allopatry model with a Bayes factor of 4.02 or a posterior probability of 0.80 (fig. 2).

Since the IM model could not be ruled out completely (posterior probability of 0.20), we evaluated whether, in this model, complete isolation was established earlier at Z-linked than autosomal loci ($T_{iso\_auto} < T_{iso\_Z}$), as expected for ecological speciation. We found no evidence for a difference in the time of isolation, with the mode of the joint posterior distribution of T_{iso_auto} and T_{iso_Z} suggesting that complete isolation was reached almost simultaneously at all loci around 1.75×10^5 generations ago (fig. 4). In addition, the joint-posterior appears symmetric with the total-posterior probability, supporting $T_{iso\_auto} < T_{iso\_Z}$ mounting to 0.52 (fig. 4).

Model fit: We used two complementary approaches to test if the studied models are capable of reproducing the observed data. Firstly, we used the feature of ABCtoolbox to contrast the marginal density of the observed data against the distribution of the marginal densities of all retained simulations to compute a p-value on the basis of which models with a poor fit can be rejected. We obtained large p-values (>0.95) for

both models. Secondly, we checked if the models were capable of generating the observed data by determining the quantile of the observed partial least square (PLS) components within the distribution of retained PLS components for each model. We found all observed PLS components to fall between the 0.24 and 0.87 quantiles.

Parameter estimates: We obtained similar estimates for most parameters under both models (table 1, fig. 5, supplementary fig. S4). One expected exception was that the estimates of the divergence time were more recent in the strict allopatry model than in the IM model. However, the time of complete isolation in the IM model was almost the same as the divergence time in the strictly allopatric model (~200,000 generations ago, corresponding to ~0.5 MA).

Although we had little power to estimate the ancestral population size under the IM model (supplementary fig. S5), it was relatively large under the preferred strict allopatry model (mode= ~100,000, table 1 & fig. 5). Both models inferred the female effective population size of *C. vinaceus* (mode= ~310,000) to be almost eight times larger than that of *C. formosanus* (mode= ~40,000, table1 and fig. 5). While there is no census data available for these species, the difference in population sizes between the two species is in perfect agreement with the much larger range of *C. vinaceus* (supplementary fig. S2). Furthermore, both models suggested that the female effective population size was roughly five times larger than the male and the total posterior probabilities for the quotient of male and female effective population sizes to be larger than one ($N_{f\_rel\_m} > 1$) were 0.90 and 0.79 in the strict allopatry and IM models, respectively.

Restricting the analysis to intronic loci: Since 18 out of the 25 autosomal and 6 out of the 10 Z-linked loci were exons, we verified if our conclusions about the speciation

of rosefinches were affected by the inclusion of coding loci in our analysis. We thus repeated all analysis to a restricted data set containing only the 7 autosomal and 4 Z-linked intronic loci and using a fresh set of 2.5×10^5 simulations per model. Despite the much reduced data set, we found overwhelming support for a purely allopatric model with a Bayes factor of 46.10 (a posterior probability of 0.98). Just as with the full data set, we did also not find any evidence for a difference in the time of isolation between autosomal and Z-linked loci, with the total-posterior probability supporting $T_{iso\_auto} < T_{iso\_Z}$ at 0.47 and the mode of the joint posterior distribution of T_{iso_auto} and T_{iso_Z} suggesting that complete isolation was reached almost simultaneously at all loci around 2.5×10^5 generations ago, only slightly older than what we obtained with the full data set (supplementary fig. S6). While slightly broader on average, the marginal posterior distributions of the remaining parameters do not differ qualitatively from those obtained with the full data set for most parameters (supplementary fig. S7). The only exception is that the introns seem to suggest a smaller ancestral population size.

Discussion

Here we proposed a novel ABC pipeline to infer the geographic mode of speciation by contrasting the genetic diversity observed in auto- and gonosomes. We showed that this approach has considerable power to distinguish between a strictly allopatric and a modified isolation with migration model for parameter values relevant for many bird or mammal species. Further, we found the approach was powerful to detect differences in isolation times between auto- and gonosomal markers, suggesting that such an approach could help to better characterize the role of ecologically divergent selection during speciation in species known to harbor many genes involved in male sexual traits and female preferences on a sex chromosome, which is particularly true

for several bird species (e.g. Sæther et al. 2007, Pyrke 2010). Finally, the proposed models allow to estimate the ratio of male to female effective population sizes, which gives an indication of the relative strength of sexual or natural selection acting on the two sexes.

Allopatric speciation of C. vinaceous and C. formosanus

Our ABC analysis suggests that extant genetic polymorphisms of *C. vinaceus* and *C. formosanus* at autosomal and Z-linked loci are more likely to have arisen from a strictly allopatric speciation model than from isolation with migration. Even the less supported IM model did not show any evidence of earlier isolation in Z-linked than in autosomal loci, an expected pattern for ecologically divergent speciation in non-allopatry.

Allopatric speciation of the two rosefinches is also supported by a low level of morphological differentiation between them. Theoretical works predict that ecologically divergent selection is more likely to lead to reproductive isolation when accompanied by assortative mating (Dieckmann and Doebeli 1999; Doebeli and Dieckmann 2000; Schluter and Conte 2009). Furthermore, reinforcement in a contact zone would further promote differentiation of sexually selected traits and female preferences (prezygotic isolation) between two diverging populations (reviewed by Coyne and Orr 2004; Price 2007). Consequently, sister species are likely to have sexually selected traits that are highly differentiated if they diverge in parapatry or sympatry (Butlin 1987; Liou and Price 1994; Kirkpatrick 2001; Ritchie 2007). In birds, male plumages and songs are major cues for species recognition and female choice (reviewed by Price 1998; Edwards et al. 2005; Seddon et al. 2008). These traits have been found to be highly differentiated between species that diverge with gene

flow (Carling et al. 2010; Li et al. 2010; Storchová et al. 2010). Although information on vocalization of *C. vinaceus* and *C. fomosanus* is not available, their male plumages are extremely similar and they have only recently been recognized as cryptic species mainly based on molecular data (Wu et al. 2011). This suggests that during the speciation of the two rosefinches, there might have been no significant reinforcement to shift sexually selected traits, which may further support their strictly allopatric speciation.

The glaciations that occurred approximately 0.42 and 0.63 million years ago probably caused the most severe sea level lowering in the last three million years (Bintanja et al. 2005; Herbert et al. 2010). These two periods might have provided the best chances of (re)connecting the montane biota in the Himalayas /eastern Tibet and Taiwan. The divergence time between *C. vinaceus* and *C. formosanus* estimated in this study was 0.5 million years ago, consistent with these two glacial periods. However, it is more recent than a previous estimate based on the mitochondrial DNA (1.7 million years ago, Wu et al. 2011). Considering a much warmer climate in the early Pleistocene (Bintanja et al. 2005; Herbert et al. 2010), we believe that the estimate based on the mitochondrial data alone might be an overestimate, a pattern that has been documented in other studies with multi-locus data (Li et al. 2010; Storchová et al. 2010; Yeung et al. 2011). Such discrepancies might be partly due to the exclusive monophyly of the two rosefinches in their mtDNA-based phylogenetic tree: time of the most recent common ancestor of the two monophyletic groups always predates their divergence time.

Our results might also infer that historical land bridges between mainland and continental islands could have different effects on the speciation of lowland and

highland species. For lowland mainland-island sister species, such as hwameis in Taiwan and the southeastern China (Li et al. 2010), the land bridges formed during glacial periods probably provided good opportunities for secondary contacts and introgressions between diverging populations to occur. By contrast, despite the availability of land bridges, secondary contacts between mainland and island populations of highland species such as the rosefinches might have largely been prohibited by unsuitable lowland habitats - a hypothesis consistent with our results. We would therefore expect ecological speciation with gene flow to be prominent in the diversification of lowland species and allopatric speciation to predominate for highland species. More studies on speciation of both highland and lowland species could lead to a better understanding of the roles of altitudinal distribution on geography and ecology in speciation.

Sex-biased ratios of effective population size

An interesting result of our ABC analysis was that the long-term effective population sizes were estimated to be approximately five times lower in males than that in females for the two rosefinches. Since these estimates are based on a lower genetic diversity at Z-linked loci, different evolutionary forces might lead to such a finding, including stronger background selection or a higher rate or selective sweeps on the Z chromosome, potentially due to hemizygosity. However, since we did not find any indication that Z-linked loci were under stronger divergent selection than autosomes in our ABC analysis, attributing the reduced diversity found at Z-linked loci to selection only seems unlikely. Assuming that sex ratios of the two rosefinches are approximately one to one as in other *Carpodacus* finches (e.g. *C. mexicanus*, Badyaev et al. 2002), the small male effective population sizes thus imply that reproductive

success of male *C. vinaceus* and *C. formosanus* could be highly skewed (Hedrick 2005).

It is possible that natural selection through predation contributes to the low male effective population size since bright and conspicuous plumage and display behaviors are known to significantly increase predation risk of males compared to cryptic females in other finch species (e.g. the chaffinches *Firngilla coelebs*, Götmark et al. 1997) and many other bird species (reviewed by Magnhagen 1991). However, high variance in male reproductive success is more often associated with strong sexual selection (e.g. Wade 1979). Although mating systems of both *C. vinaceus* and *C. formosanus* have not been studied systematically, their strong sexual dichromatism implies that males are likely to be subject of strong sexual selection as are other sexually dichromatic birds (e.g. Owens and Hartley 1998). Furthermore, avian species with more colorful plumage tend to have higher levels of extra-pair paternity (e.g. Møller and Birkhead 1994). Because males with higher pair-bond paternity are usually more likely to gain extra-pair paternity (e.g. Byers et al. 2004; Balenger et al. 2009), extra-pair fertilization could further increase the variance in male reproductive success. Extra-pair fertilization has been documented in two well-studied rosefinch species, *C. mexicanus* (e.g. Hill et al. 1994) and *C. erythrinus* (Albrecht et al. 2007; Albrecht et al. 2009). Thus, extra-pair mating is likely to be another factor contributing to the variance in male reproductive success of the two rosefinches.

Different variance in reproductive success between sexes can be used to infer intensity of sexual selection, i.e. larger variance in one sex usually indicates a stronger selection upon that sex (Bateman 1948). However, measuring variances of reproductive success between sexes in a species is not a trivial task. It requires explicit

tracking of fitness of each individual within a population (e.g. Clutton-Brock 1988). Here, we demonstrate an approach to estimate long-term effective sex ratio that might provide estimates similar to the different variance in reproductive success between sexes using a coalescent-based analysis of genetic polymorphism. It might therefore provide an alternative and convenient way to estimate the intensity of sexual selection for quantitatively comparative studies of sexual selection intensity across species.

Materials and methods

Sample collection, DNA preparation and sex identification

Blood, liver or muscle samples were collected from 25 *C. vinaceus* individuals (19 males and six females) and from 29 *C. formosanus* individuals (20 males and nine females) from different localities throughout the range (supplementary fig. S2). One male *C. nipalensis* was used as the outgroup. Samples were soaked in 100% ethanol when collected and were preserved in a -80˚C freezer before DNA extraction. Gross genomic DNA was extracted following a modified chloroform and LiCl precipitation protocol (Gemmell and Akiyama 1996). Sexes of adults were identified from their sexually dimorphic plumage coloration, and for juveniles we used molecular sex typing (Fridolfsson and Ellegren 1999).

PCR amplification, sequencing and haplotype phasing

We amplified 25 autosomal loci (18 exons and 7 introns) and 10 Z-linked loci (six exons and four introns) (supplementary table S1) by polymerase chain reactions (PCRs). Sequences of PCR primers used in this study are available in the supplementary Table S6. All PCRs were performed in a 12.5 μl reaction volume containing approximately 50 ng DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.5

mM dNTP, 0.2 μM of each primer, 1.5 mM $MgCl_2$, and 0.4 U of *Taq* DNA polymerase (Amersham Biosciences). The PCR profile for each locus consisted of 2 min at 94 ^{o}C, followed by 40 cycles of 30 s at 94^{o}C, 30 s at 53^{o}C and 1.5 min at 72 ^{o}C, and a final extension of 2 min at 72 ^{o}C by an iCycler Thermal Cycler (Bio-Rad Corp., Hercules, CA, USA). Both strands of the amplicon were sequenced with the same PCR primers using the Bigdye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA, USA) and were electrophoresed on an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA). Sequences were aligned by Sequencher 4.7 (GeneCodes Corp., Ann Arbor, MI, USA), and proofread by eye. We removed a total of 60 indels (41 and 19 for autosomal and Z-linked introns respectively) from subsequent analysis. A total of 21,512 bp (16,196 and 5,316 for autosomal and Z-linked loci respectively) of sequence was used for further analysis.

For all loci, haplotypes were reconstructed by PHASE (Stephens et al. 2001; Stephens and Scheet 2005) implemented in DnaSP v5 (Librado and Rozas 2009) with MCMC options of 10^4 iterations, thinning interval as 100 and burn-in of the first 10^3 iterations. Individuals with haplotypes determined by PHASE with a probability more than 60% were retained for subsequent analyses (Harrigan et al. 2008).

Locus-based genetic polymorphism and neutrality tests

For each locus, the numbers of polymorphic sites *S* and haplotypes *H*, haplotype diversity *Hd* (Nei 1987), average pairwise differences (or nucleotide diversity, π, Nei 1987) and Watterson's θ_w (Watterson 1975) were calculated for each of the two rosefinches using DnaSP. We also used DnaSP to count the numbers of fixed and shared mutations between the two rosefinches and Arlequin 3 (Excoffier and Lischer 2010) to calculate the two species' genetic divergence index F_{ST} (Hudson et al. 1992)

and inbreeding coefficient F_{IS}. The statistical significance of polymorphisms contributed by species, chromosomes and gene regions was examined with a general linear model using PASW Statistics 18 (IBM/SPSS, Chicago IL, USA). Multilocus neutrality was examined by the Hudson-Kreitman-Aguadé (HKA) test (Hudson et al. 1987) using the HKA program (available at http://lifesci.rutgers.edu/~heylab/). Statistical significances of χ^2 values for the HKA test were determined by comparison with a distribution from 10^4 coalescent simulations. Neutrality of each locus was also tested using two additional statistics, Tajima's *D* (Tajima 1989) and Fu and Li's *D* (Fu and Li 1993), using DnaSP.

Mutation rates and generation time

Following Li *et al*. (2010), we used divergence patterns from the outgroup (*C. nipalensis*) to estimate the substitution rate of each nuclear gene, and calibrated them using an established molecular clock for the passerine mitochondrial cytochrome b gene (Weir & Schluter 2008). The inferred rates were slightly higher for the Z-linked (mean rate ± SE = $1.98\pm0.19\times10^{-9}$ per site per year) than for the autosomal loci (mean rate = $1.53\pm0.21\times10^{-9}$ per site per year (supplementary table S2). To transform these rates into mutation rates per generation we assumed that rosefinches have an average generation time of 2.5 years (Nelson 1966, 1978; Nolan 1978; Sibley and Ahlquist 1990; Wakeley and Hey 1997).

ABC pipeline

We used an Approximate Bayesian Computation framework (Tavaré et al. 1997; Beaumont et al. 2002; Bertorelle et al. 2010) to contrast the two competing speciation models and to estimate demographic parameters for the preferred model. Here we

follow the approach introduced by Leuenberger and Wegmann (2010) as implemented in the software ABCtoolbox (Wegmann et al. 2010).

Simulations and prior distributions

All model parameters were estimated with uniform prior distributions on the $\log_{10}$ scale (table 1). Ranges of the prior distributions were chosen to be reasonably wide but guided by our previous experience where possible (e.g. Li et al. 2010; Yeung et al. 2011). For example, we assumed the upper bound of effective size to be five times larger than the mean effective size estimated from θ_w (approximately 2×10^5). The ratio of female to male effective population size was set between 1:10 and 10:1. To allow for a wide range of speciation scenarios, prior limits on the time of divergence and the rate of symmetric gene flow ($2Nm$) were set to 2×10^4 and 5×10^6 generations and 0.1 and 50 individuals per generation, respectively.

While all coalescent simulations were carried out using Fastsimcoal (Excoffier and Foll 2011), we used ABCtoolbox to choose parameter values from prior distributions and to transform them into the appropriate values requested by Fastcimcoal. We generated a total of 1.05×10^6 simulations for each model and retained a random subset of 1×10^6 simulations among those that resulted in polymorphic genetic data in each population for each marker set (only 0.03% did not).

Summary statistics for parameter inference

The choice of summary statistics is crucial for ABC analysis: having too few summary statistics will probably mean missing important information and having too many can introduce substantial noise to the data. While the model-fitting step is quite robust in this respect (Leuenberger and Wegmann 2010), extracting meaningful

information becomes hard in high dimensions of summary statistics ("curse of dimensionality", Beaumont et al. 2002; Wegmann et al. 2010). Several methods have been introduced for choosing suitable statistics (e.g. Nunes and Balding 2010; Wegmann et al. 2010; Aeschbacher et al. 2012), and the most suitable method probably depends on the underlying model and the available data.

Here, we first computed 74 summary statistics assumed to be informative about the model parameters, and then applied the widely used partial least-squares (PLS) approach (Wegmann et al. 2010) to choose informative and uncorrelated linear combinations for parameter inference, and developed a greedy search to find an informative subset of summary statistics for model choice (see below).

The initial pool of summary statistics included the standard population genetic statistics *S* (the number of polymorphic sites), *prS* (the number of private segregating sites), π (average pairwise differences) and Tajima's *D*, each of which we calculated individually for each population and marker set (e.g. S_{vin}^{auto}). We then also included several transformations of these statistics that we believe to be informative about several of the models' parameters: the sum over both marker sets per population (e.g. $S_{vin} = S_{vin}^{auto} + S_{vin}^{Z}$), the sum over both populations per marker set (e.g. $S^{auto} = S_{vin}^{auto} + S_{form}^{auto}$), the quotient of the population marker sets (e.g. $S_{vin}^{auto}/S_{vin}^{Z}$), the quotient of the sums over marker sets (e.g. S_{vin}/S_{form}) and the quotient of the sums over population (e.g. S^{auto}/S^{Z}). We additionally computed several quotients of prS and S: for each population and marker set individually (e.g. $prS_{vin}^{auto}/S_{vin}^{auto}$), for sums computed over marker sets (e.g. prS_{vin}/S_{vin}) and for sums computed over populations (e.g. prS^{auto}/S^{auto}). We further included both F_{ST} and $\pi_{vin,form}$ (average population pairwise differences) computed for each marker set (e.g. F_{ST}^{auto}),

as well as the quotient of these statistics for the marker sets (e.g. $F_{ST}^{auto} / F_{ST}^{Z}$). Finally, we also included the natural logarithm of all quotients computed.

All basic summary statistics were calculated using the command line version of Arlequin (Excoffier and Lischer 2010), which was launched after each simulation by ABCtoolbox. We then computed all sums and quotients included in the final set. Finally, to increase linearity between parameters and summary statistics, we used the Box-Cox transformation to transform each summary statistics individually following Wegmann et al. (2009). We then used the linear combinations of these summary statistics, defined by extracting model specific PLS components from a random subset of 10,000 simulations using the R-package pls (Mevik and Wehrens 2007). Based on a leave-one-out procedure, we chose to use the first six PLS components for both models (supplementary fig. S5). However, using either 5 or 7 PLS components had only little impact on the obtained posterior distributions (supplementary figs. S4 and S8).

Summary statistics for model choice

Choosing summary statistics for model choice is difficult because exactly the same set of summary statistics has to be used for both models to make their marginal densities comparable. Unfortunately, the number of possible subsets is extremely large ($>10^{22}$ in our case), rendering an exhaustive search impossible. Recently, Clegg and Owens (2002) introduced a greedy search in which they first ordered all summary statistics by how much their median values differed between the models, and then, following that order, added one summary statistic at a time until the power to distinguish between models stopped increasing.

Here, we implemented a similar yet more rigorous approach to find a suitable subset of summary statistics for model choice, without the restriction that the statistics must be added in a specific order. Rather, our greedy search performed the following three steps in each iteration:

1) For each subset of summary statistics with unknown power to distinguish between models, run ABC model choice as outlined below on 500 pseudo-observed data sets (PODS, data sets generated with known parameter values drawn from the prior) for each model, and estimate power as the fraction of cases in which the preferred model was the model used to generate the data.

2) From the current collection of subsets, choose the 12 subsets with the highest power.

3) Create new, additional subsets by extending a selected subset with a single summary statistic that is not strongly correlated with any other summary statistic in the subset (correlation coefficient <0.95). Do that for each selected subset and each possible, additional summary statistic.

We initiated our search with all pairs of summary statistics, and ran the search until no new subsets were added. We performed five independent runs of this search to check for convergence. In three out of these five runs exactly the same subset of eight summary statistics resulted in the highest power (0.71): $\log\left(\frac{\mathrm{prS}^{\mathrm{auto}}}{prS^{Z}}\right)$, $\log\left(\frac{prS_{vin}}{S_{vin}}\right)$, $\log\left(\frac{prS_{form}}{S_{form}}\right)$, D_{vin}, D_{form}, $\log\left(\frac{\pi_{vin}^{auto}}{\pi_{vin}^{Z}}\right)$, ${F_{ST}}^{Z}$, $\log\left(\frac{{\pi_{vin,form}}^{auto}}{{\pi_{vin,form}}^{Z}}\right)$. The other two runs resulted in subsets that differed in a single statistic, and that statistic replaced one highly correlated with it: ${F_{ST}}^{auto}$ instead of ${F_{ST}}^{Z}$ and

$\log\left(\frac{\pi_{form}^{auto}}{\pi_{form}^{Z}}\right)$ instead of $\log\left(\frac{\pi_{vin}^{auto}}{\pi_{vin}^{Z}}\right)$, respectively.

Inferring posterior distributions and choosing between the models

We inferred posterior distributions and marginal densities by retaining the 5×10^4 simulations closest to the observed data, by fitting a local likelihood model to the parameter values and summary statistics of these simulations and by estimating truncated prior densities from the retained parameter values. Fitting a likelihood model requires specific assumptions to be made about the form of the local likelihood function, and hence potentially introduces an additional source of approximation. Here we follow Leuenberger and Wegmann (2010), and assume that a general linear model (GLM) can be adequately fitted to the local likelihood function. The choice of a GLM was admittedly motivated by the availability of analytical solutions to obtain posterior densities (Leuenberger and Wegmann 2010) and their implementation in the software ABCtoolbox which we used here. In order to increase the linearity of the relationship between parameters and statistics, we followed Wegmann et al. (2009), and individually Box-Cox transformed each statistic. In addition, the PLS components we use for parameter inference are linear combinations of summary statistics defined using a linear regression framework.

Estimating joint posterior surfaces supporting $T_{iso\_auto} < T_{iso\_Z}$

We used a Markov chain Monte Carlo method (MCMC) implemented in the software ABCtoolbox to generate 10^4 samples from the joint posterior of T_{iso_auto} and T_{iso_Z} to estimate the total joint posterior surface supporting $T_{iso\_auto} < T_{iso\_Z}$ as the fraction of samples for which $T_{iso\_auto} < T_{iso\_Z}$. All MCMC chains were started at the joint posterior mode, and the first 10^3 steps (burnin) were discarded. In each step, both

parameters were updated with a uniform transition kernel with the width of one standard deviation of random samples of the marginal posterior distribution, and we only recorded every ten steps.

Validating model choice

We validated our ABC model choice by empirically calibrating obtained Bayes Factors using PODS (Peter et al. 2010). In our ABC setting, the decision on which model (M_1 or M_2) is better supported is based on the Bayes Factor BF = $Pr(M_1)/Pr(M_2)$. For a given Bayes Factor X, the true underlying model should be M_1 with posterior probability $P_1=X/(X+1)$. In order to assess whether the BFs resulting from our model choice procedures were unbiased, we generated 5×10^4 PODS for each model with parameters drawn from the prior distribution, performed ABC model choice, and recorded the posterior probability P_1 obtained. We then allocated our data sets to 100 discrete bins of P_1, and checked within each bin whether the proportions of data sets generated under M_1 and M_2 were equal to $\widehat{P_1}$ and $1-\widehat{P_1}$, respectively, where $\widehat{P_1}$ is the center of the bin. This distribution was then used to correct empirically for any bias found in the ABC posterior probabilities.

Validating parameter estimates

We validated our marginal posterior distributions by estimating posterior distributions for 1×10^4 PODS for each model, and for each marginal posterior distribution recorded the smallest continuous highest posterior density interval (HPD) that included the true parameter value. If our posterior distributions were well calibrated, an HPD covering a fraction *p* of the total posterior surface would be expected to harbor the true parameter values in a fraction *p* of the total number of PODS used (Wegmann et al.

2009). As a consequence, the distribution of the smallest continuous HPDs over all PODS for a given parameter is expected to be uniform, a characteristic we tested individually for each marginal posterior distribution.

Power to detect difference in isolation times

We estimated the power of our ABC approach to detect differences in isolation times between autosomal and Z-linked loci by generating 10^4 PODS in which we sampled isolation times from their respective prior distributions, but fixed all other parameters to the values at the mode of the marginal posterior distribution obtained under the IM model. For each of these PODS we then inferred the joint posterior distribution of T_{iso_auto} and $T_{iso\_Z,}$ and recorded the total posterior probability supporting $T_{iso\_auto} < T_{iso\_Z}$. We then calculated the median of these probabilities for each cell of a 50 × 50 grid across the joint parameter space for T_{iso_auto} and T_{iso_Z}, and used these to plot a filled contour using R.

Acknowledgements

We are grateful to Chung-Wei Yen and Yen-Jun Chen of the National Museum of Natural Science of Taiwan who kindly provided tissue samples. Special thanks are given to Alan Watson, Chih-Ming Hung, Carol K-L Yeung and Claudia Bank who read an early version of this manuscript and provided many insightful comments. This work was supported by the National Science Council (NSC) of Taiwan (SHL: NSC99-2628-B-003-001-MY3), the National Natural Science Foundation of China (FML 30925008) and the Swiss National Science Foundation (142643). JHC is a post-doctoral researcher funded by the NSC (grand number

NSC101-2811-B-003-005). Our gratitude also goes to the National Taiwan Normal University (NTNU) and the Academic Paper Editing Clinic, NTNU for supporting the charge for paper publication and English editing respectively.

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Table 1. Characteristics of marginal prior and posterior distributions[a].

	Prior		Allopatry (posterior probability 80.1%)					IM (posterior probability 19.9%)				
				50% HPD[b]		95% HPD[b]			50% HPD[b]		95% HPD[b]	
	lower	upper	Mode	lower	upper	lower	upper	Mode	lower	upper	lower	upper
$\log_{10}(N_{anc})$	3.70	6.00	5.04	4.77	5.27	4.10	5.61	4.99	4.31	5.31	3.76	5.84
$\log_{10}(N_{form})$	3.70	6.00	4.60	4.49	4.71	4.23	4.90	4.60	4.43	4.73	4.00	4.92
$\log_{10}(N_{vin})$	3.70	6.00	5.49	5.33	5.58	5.04	5.79	5.39	5.21	5.53	4.77	5.74
$\log_{10}(N_{f\_rel\_m})$	-1.00	1.00	0.67	0.40	0.84	-0.18	0.99	0.72	0.35	0.89	-0.48	0.99
$\log_{10}(T_{div})$	4.30	6.70	5.34	5.21	5.46	4.99	5.74	6.31	5.98	6.53	5.39	6.69
$\log_{10}(T_{iso}^{auto})$	2.00	6.70	-	-	-	-	-	5.20	4.77	5.67	3.54	6.31
$\log_{10}(T_{iso}^{Z})$	2.00	6.70	-	-	-	-	-	5.26	4.78	5.67	3.51	6.40
$\log_{10}(2Nm)$	-1.00	1.70	-	-	-	-	-	-0.72	-0.95	-0.29	-0.99	1.28

[a] The posterior distributions are shown in fig. 4 and fig. 5 and supplementary fig. S4.

[b] The highest posterior density interval

Figure Legends

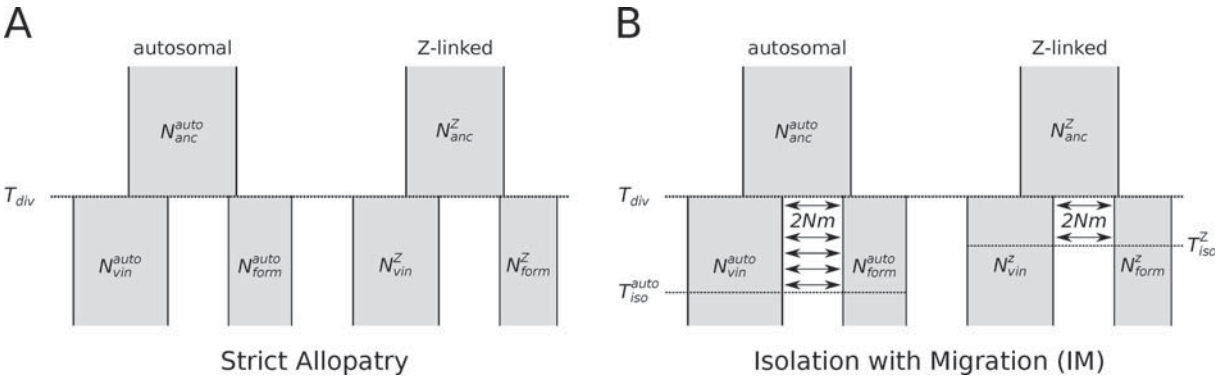
FIG. 1. Speciation models. We contrasted two competing speciation models: strict allopatry (A) and isolation with migration (B), both taking the differences in autosomal and Z-linked markers fully into account. See text for more details on parameterization.

FIG. 2. Validation of model choice. A comparison of the posterior probability in favor of a strictly allopatric speciation as estimated via ABC against an empirical estimate of the same probability obtained through simulations (see Materials and Methods) reveals that our ABC approach for model choice is relatively well calibrated, particularly when the support for a given model is estimated to be strong. The dashed lines indicate the translation of our observed ABC posterior probability into an empirically corrected posterior probability.

FIG. 3. Power to distinguish isolation times. The power to infer a difference in the times at which complete isolation was established at autosomal and Z-linked markers as measured by the total surface of the joint posterior supporting an earlier isolation at Z-linked loci ($A_{Z<auto}$). Shown is the distribution of median $A_{Z<auto}$ values obtained for different combinations of isolation times when fixing all parameters to the modal values estimated from the rosefinch data set.

FIG. 4. Two dimensional posterior distribution for the time points at which complete isolation was established at autosomal and Z-linked loci under an isolation-with-migration (IM) model. The mode is shown as a black dot and highest posterior density intervals as contour lines.

FIG. 5. Marginal posterior distribution of demographic parameters. Shown are the marginal posterior distributions for all parameters of the strict allopatric model on a $\log_{10}$ scale. The horizontal, dotted line corresponds to the prior distribution, which was bounded within the range shown. Characteristics of both prior and posterior distributions are found in table 1.The posterior distributions for the isolation-with-migration (IM) model are reported in supplementary fig. S4.


A
autosomal
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T_{div}
N_{vin}^{auto}
N_{form}^{auto}
N_{vin}^{Z}
N_{form}^{Z}
Strict Allopatry
B
autosomal
Z-linked
N_{anc}^{auto}
N_{anc}^{Z}
T_{div}
2Nm
2Nm
T_{iso}^{Z}
N_{vin}^{auto}
N_{form}^{auto}
T_{iso}^{auto}
N_{vin}^{Z}
N_{form}^{Z}
Isolation with Migration (IM)

FIG. 1.

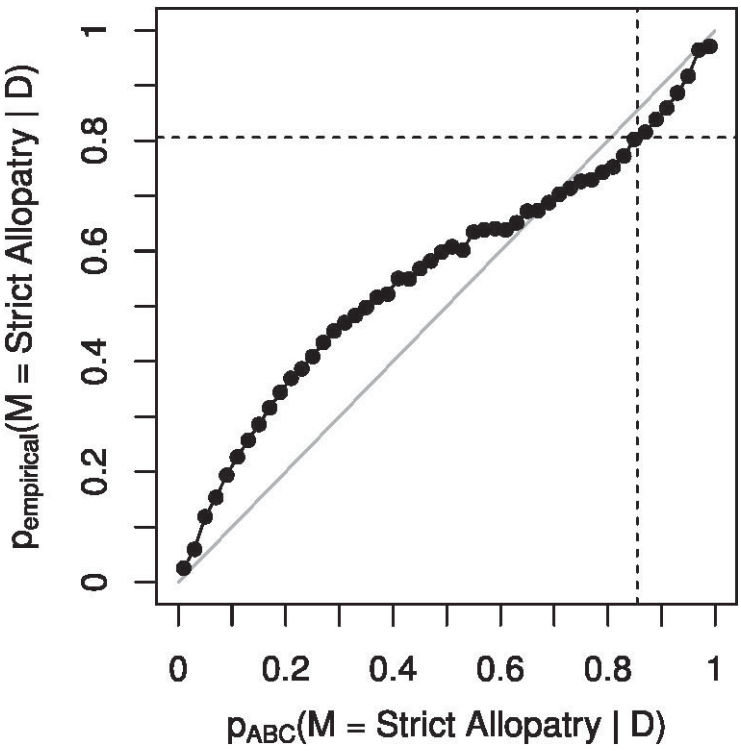
$p_{empirical}$(M = Strict Allopatry | D)
0
0.2
0.4
0.6
0.8
1
p_{ABC}(M = Strict Allopatry | D)

FIG. 2.

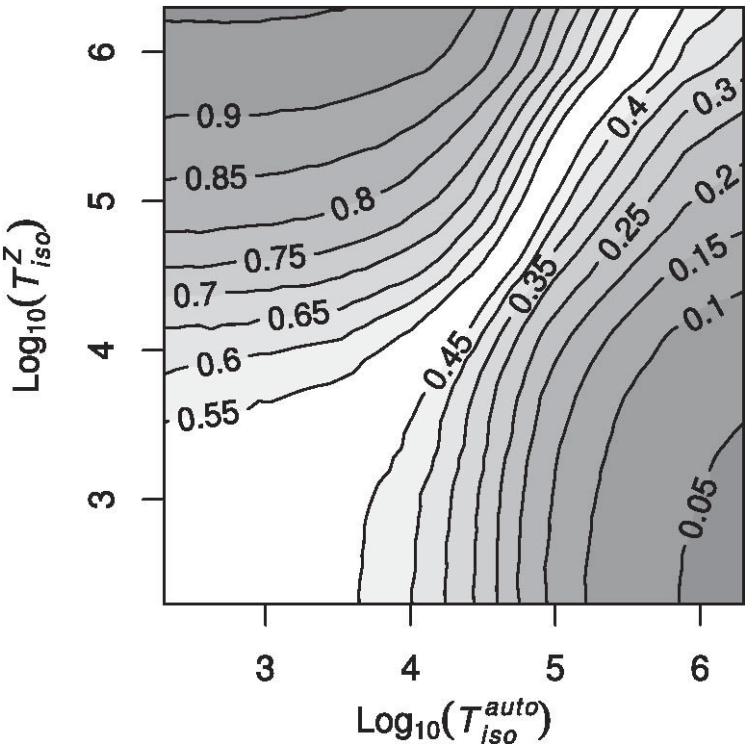
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.45
0.4
0.35
0.25
0.15
0.1
0.05
3
4
5
6
$\mathrm{Log}_{10}(T_{iso}^{Z})$
$\mathrm{Log}_{10}(T_{iso}^{auto})$

FIG. 3.

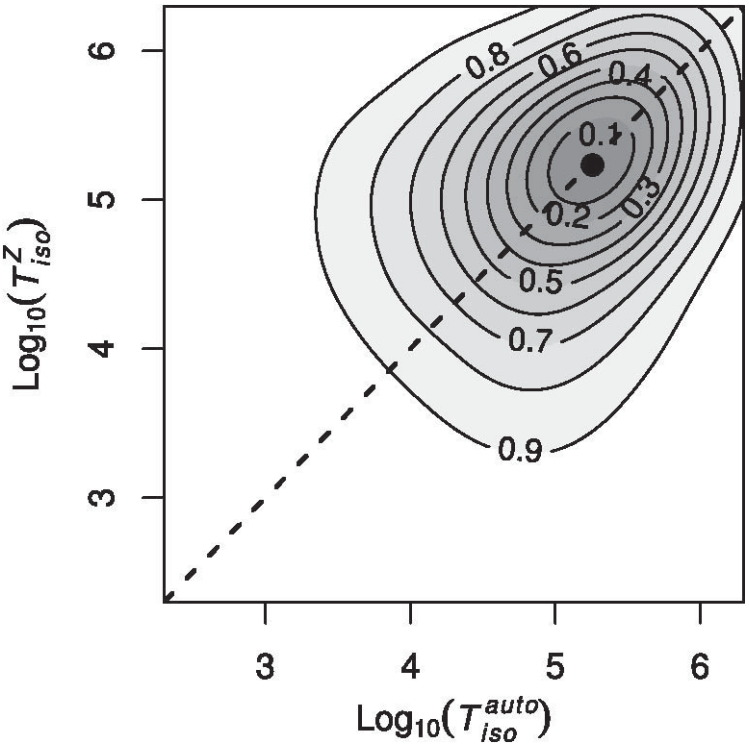
0.8
0.6
0.4
0.1
0.3
0.2
0.5
0.7
0.9
3
4
5
6
$\mathrm{Log}_{10}(T_{iso}^{Z})$
$\mathrm{Log}_{10}(T_{iso}^{auto})$

FIG. 4.

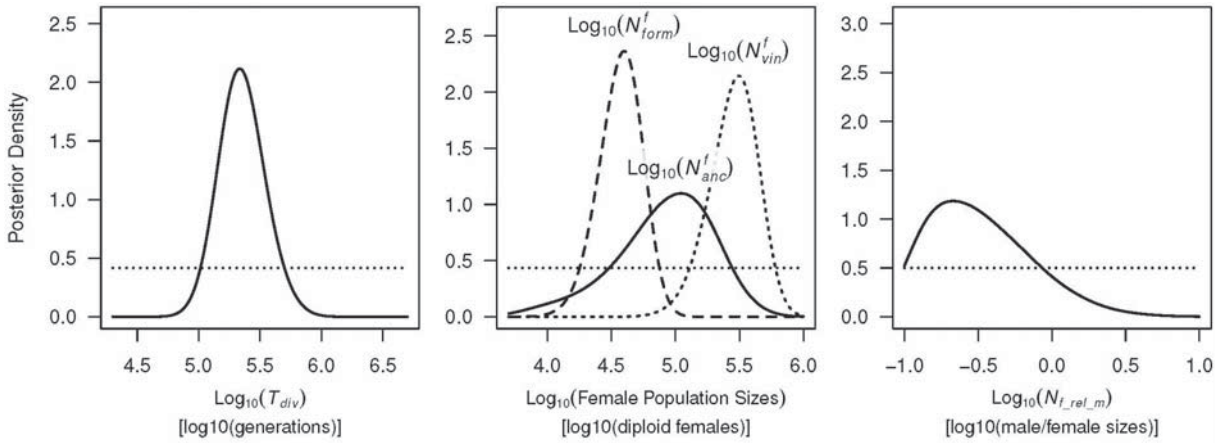
Posterior Density
2.5
2.0
1.5
1.0
0.5
0.0
4.5
5.0
5.5
6.0
6.5
Log10(Tdiv)
[log10(generations)]
Log10(N^f_form)
Log10(N^f_vin)
Log10(N^f_anc)
4.0
4.5
5.0
5.5
6.0
Log10(Female Population Sizes)
[log10(diploid females)]
3.0
−1.0
−0.5
0.0
0.5
1.0
Log10(Nf_rel_m)
[log10(male/female sizes)]

FIG. 5.