Bayesian inference of introgression between sister lineages using genomic data

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Inference of interspecific gene flow using genomic data is important to reliable reconstruction of species phylogenies and to our understanding of the speciation process. Gene flow is harder to detect if it involves sister lineages than nonsisters; for example, most heuristic methods based on data summaries are unable to infer gene flow between sisters. Likelihood-based methods can identify introgression between sisters but the test exhibits several nonstandard features, including boundary problems, indeterminate parameters, and multiple routes from the alternative to the null hypotheses. In the Bayesian test, those irregularities pose challenges to the use of the Savage-Dickey (S-D) density ratio to calculate the Bayes factor. Here we develop a theory for applying the S-D approach under nonstandard conditions. We show that the Bayesian test of introgression between sister lineages has low false-positive rates and high power. We discuss issues surrounding the estimation of the rate of gene flow, especially at very low or very high rates. We find that the species split time has a major impact on the information content in the data, with more information at deeper divergence. We use a genomic dataset from *Sceloporus* lizards to illustrate the test of gene flow between sister lineages.

Keywords: Bayes factor, BPP, introgression, multispecies coalescent, Savage-Dickey density ratio

Introduction

Genomic data can be used to test introgression between sister species

Detecting gene flow between species and estimating the rate of gene flow is important to reconstructing species phylogenies and testing theories of speciation (Sousa and Hey, 2013; Payseur and Rieseberg, 2016; Roux *et al.*, 2016). A number of methods have been developed to infer gene flow using genomic data, including heuristic methods based on data summaries and likelihood methods based on parametric models of gene flow (Jiao *et al.*, 2021). The former class of methods includes the D-statistic (or ABBA-BABA test) (Green *et al.*, 2010), which uses genome-wide site-pattern counts in a species quartet; SNaQ (Solis-Lemus and Ane, 2016; Solis-Lemus *et al.*, 2017; see also Yu *et al.*, 2012) which uses reconstructed gene tree topologies; and ∂a∂i (Gutenkunst *et al.*, 2009) and FASTSIMCOAL2 (Excoffier *et al.*, 2013), which use the joint site frequency spectrum at single nucleotide polymorphism (SNP) sites. The latter class of methods, including both maximum likelihood (ML) and Bayesian inference, has the major feature that they use the multispecies coalescent (MSC) model with gene flow to average over genealogical histories underlying sequences sampled at each locus (Jiao *et al.*, 2021). In ML, this is achieved through numerical integration (Zhu and Yang, 2012; Dalquen *et al.*, 2017), while in Bayesian inference, it is through Markov chain Monte Carlo (MCMC) algorithms. Gene flow has been modelled as either a discrete introgression/hybridization event which occurred at a particular time point in the past (the MSC-I model; e.g., PhyloNet, Wen and Nakhleh, 2018; BPP, Flouri *et al.*, 2020) or as a continuous process with a constant migration rate per generation (the MSC-M model; IMa3, Hey *et al.*, 2018; BPP, Flouri *et al.*, 2023). Our main focus in this paper is inference under the discrete introgression model (MSC-I) in the Bayesian framework.

Introgression may be harder to detect if it involves sister lineages than nonsisters. For example, gene flow between nonsisters may cause asymmetries in the gene tree distribution and in site-pattern counts in genomic data for a species quartet, while gene flow between sisters does not. As a result, quartet summary methods such

as D and SNaQ, which rely on counts of estimated gene trees or site patterns for species quartets, can detect gene flow between nonsisters but not between sisters. Likelihood methods use information in the coalescent times or branch lengths on gene trees, and in the genealogical variation across the genome, and can thus infer gene flow between sister lineages. They also accommodate multiple samples from the same species (in particular from the recipient lineage, which may boost the information in the data about gene flow considerably. In simulations, Bayesian inference under both MSC-I and MSC-M models showed similar power in inferring gene flow between sisters to that between nonsisters (Huang *et al.*, 2020; Thawornwattana *et al.*, 2023, 2025).

We note that in this context "sister species" simply means that the lineages involved in introgressive hybridization are sister branches on the phylogeny, and does not imply that the two species are closely related or there is any biological difference between introgression involving sisters and that involving nonsisters. For example, given the phylogeny $(A, (B, C))$, the $A \to B$ introgression is between nonsisters, but is between sisters if species C is not included in the data (for example, if C has gone extinct). The term thus has no biological significance. Also in this paper we use the terms species, populations, and lineages interchangeably.

The Bayes factor for testing nested hypotheses can be calculated via the Savage-Dickey (S-D) density ratio

The standard approach to model comparison in the Bayesian framework is the Bayes factor, defined as the ratio of the marginal likelihood values between the two compared models (Jeffreys, 1935). The marginal likelihood under a model is an average (often a high-dimensional integral) over the parameters under the model. This typically requires expensive computation involving many MCMC runs (Fourment *et al.*, 2020, see also Discussion). In the special case of comparing nested hypotheses, the Bayes factor may be calculated via the Savage-Dickey (S-D) density ratio (Dickey, 1971; Ji *et al.*, 2023), which involves far less computation. In phylogenetics Suchard *et al.* (2003) used the S-D density ratio to conduct Bayesian tests of the molecular clock; however, in that study the phylogeny is allowed to differ between the clock and no-clock models which appears to invalidate the S-D approach.

Let H_0 be the null hypothesis and H_1 the alternative hypothesis, with H_0 being a special case of H_1. In other words, H_1 reduces to H_0 when certain parameters of interest in H_1 take special values (called the *null value*): $\omega = \omega_0$. Suppose nuisance parameters λ exist in both models, so that the prior under H_0 is $\pi_0(\lambda)$ and that under H_1 is $\pi(\lambda, \omega) = \pi(\omega)\pi(\lambda|\omega)$. On the condition that the priors on nuisance parameters match between the two models at the null value,

$$\pi_0(\lambda) = \pi(\lambda|\omega_0), \tag{1}$$

the Bayes factor B_{10} in support of H_1 is given by

$$B_{10} = \frac{\pi(\omega_0)}{\pi(\omega_0 \mid \boldsymbol{x})}, \tag{2}$$

where $\boldsymbol{x}$ represent the data, and $\pi(\omega_0)$ and $\pi(\omega_0|\boldsymbol{x})$ are the prior and posterior densities of ω, respectively, under H_1 evaluated at $\omega = \omega_0$ (Dickey, 1971). Dickey's (1971) proof of eq. 2 accounts for possible presence of nuisance parameters (λ) but does not accommodate the presence of unidentifiable parameters. This is dealt with in the extended proof of Ji *et al.* (2023).

Irregularities of the test of introgression between sister lineages and objectives of this paper

Consider two species that diverged time τ ago with the phylogeny (A, B). The MSC model with no gene flow is the null hypothesis (H_0) with parameter τ (fig. 1a). In the alternative hypothesis H_1, introgression occurs at time τ_Y (in the past) from species A to B (or from X to Y), with the introgression probability $\varphi \equiv \varphi_{XY}$, defined as the proportion of immigrants in the recipient population Y from the donor population X (fig. 1b). Note that time runs forward when we define parameters. Both H_0 and H_1 involve a population size parameter θ, which is dropped in our notation here. The parameter vector is thus $\boldsymbol{\theta}_0 = (\tau)$ for H_0 and $\boldsymbol{\theta} = (\varphi, \tau_Y, \tau_R)$ for H_1 (fig. 1a&b).

While H_0 is clearly nested within H_1, the test comparing the two models involves several non-standard features.

1. H_0 may be represented by setting $\varphi = 0$ in H_1, but when $\varphi = 0$, the introgression time (τ_Y) becomes unidentifiable; if there is no introgression, the time of introgression is indeterminate.
2. H_1 reduces to H_0 (by fixing parameters of interest to their null values) via three routes (fig. 1c):
   (a) $\varphi = 0$ (in which case τ_Y becomes unidentifiable).
   (b) $\tau_Y = \tau_R$ (in which case φ becomes unidentifiable).
   (c) $\varphi = 1$ (in which case τ_R becomes unidentifiable, with the introgression time τ_Y becoming the species split time).

Whereas in regular cases H_0 corresponds to one point in the parameter space for H_1, here H_0 corresponds to three lines in H_1 (fig. 1d&e). Under such nonstandard conditions neither of the proofs of Dickey (1971) and Ji *et al.* (2023) works. Our first objective in this paper is thus to extend the theory for computing the Bayes factor via the S-D density ratio to nonstandard conditions. Note that the irregularities discussed here affect the use of the S-D density ratio to calculate the Bayes factor, not the Bayes factor itself. However methods for calculating the Bayes factor via marginal likelihood (which are not affected by the irregularities discussed here) involve many MCMC runs and >100 times more computation than the S-D approach (Fourment *et al.*, 2020, and see Discussion). We study simpler problems of Gaussian mixtures to develop the theory.

Figure 1f&g shows posterior summaries and trace plots for parameters in the introgression model (H_1) from a BPP analysis of a large dataset simulated under the null model of no gene flow (H_0). As the data are

informative, the MCMC spends most of the time visiting the neighborhoods of the three lines of figure 1d which represent the true model H_0. However, do there exist multiple modes on the posterior surface (as the marginal summary of φ seems to show)? How often should the chain visit the neighborhoods of the three lines? How should one summarize the MCMC sample and what biological conclusions should one draw from such posterior samples as that of figure 1f&g? Our second objective is thus to study the behavior of the MCMC algorithm when the MSC-I model is fitted to data with no or little gene flow. We characterize the parameter space and posterior surface under H_1, and the probabilities with which the MCMC visits different regions of the parameter space under H_1, when data are generated under H_0, providing guidelines on Bayesian analysis of datasets at low rates of gene flow.

Our third objective is to evaluate the power of the Bayesian test and the precision of Bayesian estimation of the introgression probability, assessing the information content in multilocus sequence data concerning introgression between sister lineages.

Our paper is structured as follows. First we develop a theory for applying the S-D approach to computing the Bayes factor for testing introgression between sister lineages. We develop and illustrate the theory through simple examples, for which numerical calculation is efficient. We prove that when multiple routes exist for reducing H_1 to H_0, the local behavior of each route can be used to calculate the Bayes factor via the S-D approach. We study the posterior surface in large datasets generated under the null model of no gene flow and conclude that the posterior probabilities for different regions of the parameter space do not converge to a point but rather to a non-degenerate probability distribution, when the data size approaches infinity. Finally we conduct a simulation study and an asymptotic analysis (treating data of infinite size) to examine the power of the Bayesian test of introgression and the precision of parameter estimation. We find that information content is greatly influenced by the species divergence time. We discuss the impact of the irregularities of the testing problem on Frequentist tests of introgression, such as the likelihood ratio test (LRT) based on composite likelihood used in ∂a∂i (Gutenkunst *et al.*, 2009) and FASTSIMCOAL2 (Excoffier *et al.*, 2013).

Theory

Savage-Dickey (S-D) density ratio under nonstandard conditions

We consider the general case first and illustrate the theory using three simple examples, before dealing with the Bayesian test of introgression between sister species.

Let the vector of parameters be $\boldsymbol{\theta}_0$ with the prior $\pi_0(\boldsymbol{\theta}_0)$ under H_0, and $\boldsymbol{\theta}$ with the prior $\pi(\boldsymbol{\theta})$ under H_1. Given data $\boldsymbol{x}$, let the likelihood be $L_0(\theta_0) = p_0(\boldsymbol{x}|\theta_0)$ under H_0 and $L(\boldsymbol{\theta}) = p(\boldsymbol{x}|\boldsymbol{\theta})$ under H_1. Here we use the subscript 0 for densities under H_0 and no subscript for those under H_1. The Bayes factor in support of H_1 over H_0 is defined as

$$B_{10} = \frac{m}{m_0} = \frac{\int \pi(\boldsymbol{\theta}) L(\boldsymbol{\theta})\, \mathrm{d}\boldsymbol{\theta}}{\int \pi_0(\boldsymbol{\theta}_0) L_0(\boldsymbol{\theta}_0)\, \mathrm{d}\boldsymbol{\theta}_0}. \tag{3}$$

We recognize three types of parameters in H_1, with $\boldsymbol{\theta} = (\omega, \boldsymbol{\lambda}, \xi)$, where ω are parameters of interest, which are constrained to formulate the null hypothesis (H_0); λ are nuisance parameters, which are shared between H_0 and H_1 and usually have the same interpretation in the two models; and ξ are unidentifiable/indeterminate parameters, which become unidentifiable when H_1 is reduced to H_0. In simple cases, H_1 reduces to H_0 when the parameters of interest take special values that represent no effect (called *null value* or *null point*), that is, $\omega = \omega_0$.

In our problems there are multiple routes from H_1 to H_0, and the partitioning of parameters, $\boldsymbol{\theta} = (\omega, \lambda, \xi)$, differs depending on the route. For example, in the test of introgression of figure 1, there are three routes: a, b, c, with the partitioning of parameters for each route shown in table 1(i), and the null point (which is a point in the parameter space of H_0) corresponds to three segments (a, b, c) in the parameter space of H_1 (fig. 1d).

We show that with multiple routes for reducing H_1 to H_0, each can be used to construct an S-D density ratio to compute B_{10}.

Theorem 1. *Suppose H_0 corresponds to multiple segments in the parameter space of H_1 and at segment j, we partition the parameters as $\boldsymbol{\theta} = (\omega_j, \lambda_j, \xi_j)$, where ω_j are parameters of interest (with $\omega_j = \omega_{j0}$ being the null value), λ_j the nuisance parameters and ξ_j the unidentifiable parameters. On the condition that*

$$\pi_0(\lambda_j) = \pi(\lambda_j|\omega_{j0}), \tag{4}$$

where $\pi(\lambda_j|\omega_{j0}) = \int \pi(\lambda_j, \xi_j|\omega_{j0})\, \mathrm{d}\xi_j$, the Bayes factor B_{10} of eq. 3 is given as

$$B_j = \frac{\pi(\omega_{j0})}{\pi(\omega_{j0}|\boldsymbol{x})} = B_{10}. \tag{5}$$

Proof. As H_0 and H_1 are nested, $L(\omega_{j0}, \lambda_j, \xi_j) = L_0(\lambda_j)$. Thus

$$\begin{aligned} B_j &= \frac{\pi(\omega_{j0})}{\iint \pi(\omega_{j0}, \lambda_j, \xi_j|\boldsymbol{x})\, \mathrm{d}\lambda_j\, \mathrm{d}\xi_j} \\ &= \frac{\pi(\omega_{j0})}{\iint \frac{1}{m}\pi(\omega_{j0})\pi(\lambda_j, \xi_j|\omega_{j0}) L(\omega_{j0}, \lambda_j, \xi_j)\, \mathrm{d}\lambda_j\, \mathrm{d}\xi_j} \\ &= \frac{m}{\iint \pi(\lambda_j, \xi_j|\omega_{j0}) L_0(\lambda_j)\, \mathrm{d}\lambda_j\, \mathrm{d}\xi_j} \\ &= \frac{m}{\int \pi_0(\lambda_j) L_0(\lambda_j)\, \mathrm{d}\lambda_j} \\ &= \frac{m}{m_0}. \end{aligned} \tag{6}$$

□

This proof resembles the reverse of the proof of Ji *et al.* (2023, eq. A1). However, here the argument concerns the local behavior of the densities around segment j.

More generally, H_0 may be specified by setting up equality constraints on parameters of interest in H_1, that

is $c(\boldsymbol{\theta}) = 0$, rather than by setting parameters of interest to the null value ($\omega = \omega_0$). For example the test for equality of two population means may be formulated as $\boldsymbol{\theta} = (\mu_1, \mu_2)$ with the constraint $c(\boldsymbol{\theta}) = \mu_1 - \mu_2 = 0$. As the parameters of interest depend on the segment, we use $\boldsymbol{\theta}$ instead of ω_j to specify constraints. Suppose there are J routes of going from H_1 to H_0. For route j, let there be K_j equality constraints, $c_j = (c_{j1}, \cdots, c_{jK_j})$, so that segment j is represented by

$$\Omega_{j0} = \left\{ \boldsymbol{\theta} : \bigcap_k c_{jk}(\boldsymbol{\theta}) = 0 \right\}. \tag{7}$$

For simplicity, we will use K instead of K_j but note that K may differ among segments. We use infinitesimals $\epsilon_{jk} > 0$, $j = 1, \cdots, J; k = 1, \cdots, K$, to define a *null region*, a subspace of the parameter space of H_1 in the neighborhood of segment j,

$$\Omega_{j,\epsilon} = \left\{ \boldsymbol{\theta} : \bigcap_k |c_{jk}(\boldsymbol{\theta})| < \epsilon_{jk} \right\}. \tag{8}$$

Let $\epsilon_j = (\epsilon_{j1}, \cdots, \epsilon_{jK})$. Define

$$\begin{aligned} \pi(\Omega_{j0}) &= \lim_{\epsilon_j \to 0} \frac{\mathbb{P}(\Omega_{j,\epsilon})}{\epsilon_{j1} \cdots \epsilon_{jK}}, \\ \pi(\Omega_{j0}|\boldsymbol{x}) &= \lim_{\epsilon_j \to 0} \frac{\mathbb{P}(\Omega_{j,\epsilon}|\boldsymbol{x})}{\epsilon_{j1} \cdots \epsilon_{jK}}, \end{aligned} \tag{9}$$

to be the prior and posterior densities for Ω_{j0} under H_1. Then

$$B_j = \frac{\pi(\Omega_{j0})}{\pi(\Omega_{j0}|\boldsymbol{x})} \approx \frac{\mathbb{P}(\Omega_{j,\epsilon})}{\mathbb{P}(\Omega_{j,\epsilon}|\boldsymbol{x})} \tag{10}$$

is an approximation of B_{10}.

In the simple case of setting parameters of interest to null values, this reduces to eq. 5, with $\pi(\Omega_{j0}|\boldsymbol{x}) = \pi(\omega_{j0}|\boldsymbol{x})$, say. In general, one may reparametrize H_1 and treat $\omega_j = c_j$ as parameters, and derive the induced prior on the new parameters through a variable transform. Then the Bayes factor for segment j of eq. 10 will take the simpler form of eq. 5. Thus we do not see any conceptual difference between eqs. 5 and 10. Henceforth we will use the notation of eq. 10, but with reparametrization used to verify the condition on priors (eq. 4) (see Remark 2 below). Note that the number of constraints K in eqs. 9&10, which may be viewed the degree of freedom for the test, equals the dimension of ω_j in eq. 5.

We will use the differentials ϵ_j to calculate $\mathbb{P}(\Omega_{j,\epsilon}|\boldsymbol{x})$ for segment j by processing an MCMC sample under H_1 (eq. 10) (Ji *et al.*, 2023). One may also use kernel density smoothing to calculate $\pi(\Omega_{j0}|\boldsymbol{x})$. The prior density $\pi(\Omega_{j0})$ is typically tractable analytically.

If the condition on priors holds for each segment, so that all segments give the same S-D density ratio, with $B_j = B_{10}$ (eqs. 5 or 10), we can use all segments to calculate a combined density ratio. Let

$$\Omega_0 = \bigcup_j \Omega_{j0}, \quad \Omega_\epsilon = \bigcup_j \Omega_{j,\epsilon} \tag{11}$$

be the *null point* (or constraints) and *null region*, respectively. Then

$$B = \frac{\pi(\Omega_0)}{\pi(\Omega_0|\boldsymbol{x})} \approx \frac{\mathbb{P}(\Omega_\epsilon)}{\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})}. \tag{12}$$

We make two observations regarding the condition on priors of eq. 4.

First, if the condition on priors (eq. 4) does not hold, a correction factor may be applied

$$B_j = \frac{\pi(\omega_{j0})}{\pi(\omega_{j0}|\boldsymbol{x})} \Big/ \mathbb{E}\left\{ \frac{\pi_0(\lambda_j)}{\pi(\lambda_j|\omega_{j0})} \right\}, \tag{13}$$

with the expectation taken over $\pi(\lambda_j|\boldsymbol{x}, \omega_{j0})$ (Verdinelli and Wasserman, 1995). However, this does not appear to be simpler to calculate than the marginal likelihood values (m_0, m), and is thus not pursued here. Instead later we discuss ideas for approximate calculation of B_{10} when the condition on priors does not hold.

Second, conditioning on intuitively equivalent events may lead to different conditional distributions. Consider for example the so-called one-sample text, the test of $H_0 : \mu = 0$ against $H_1 : \mu \neq 0$ with unknown variance σ^2. Conditioning on $\mu = 0$ or on $\xi = \mu/\sigma = 0$ leads to different distributions, with $p(\sigma^2|\mu = 0) \neq p(\sigma^2|\xi = 0)$ (see SI text 1).

This phenomenon is known as the Borel-Kolmogorov paradox and is controversial. It is known as the "equivalent-event fallacy" as seemingly equivalent events are not truly equivalent (fig. S1). One should note that the condition on priors (eq. 4) may hold for one parametrization but not another, and should check the condition when calculating the S-D density ratio. Note that the issue concerns the S-D approach to calculating the Bayes factor, not the Bayes factor itself. The marginal likelihood values are not affected by reparametrization, so that B_{10} is well-defined and is the same for the two parametrizations.

Example 1. Comparison of two population means

Consider a test of equality of two population means, μ_1 and μ_2. We have $H_0 : \mu_1 = \mu_2 = \mu$ while H_1 specifies $\mu_1 \neq \mu_2$, with the constraint $\Omega_0 : \mu_1 = \mu_2$ reducing H_1 to H_0. This does not have any irregularities. It is analytically tractable and serves to illustrate the S-D approach to calculating the Bayes factor and the use of reparametrization to deal with a null hypothesis formulated via equality constraints. Here we summarize the results, with the derivations given in SI text 2.

We assign the prior $\mu \sim N(0, 1/(2\tau))$ under H_0, and $p(\mu_1, \mu_2) = \phi(\mu_1; 0, 1/\tau)\phi(\mu_2; 0, 1/\tau)$ under H_1, where $\phi(y; \mu, \sigma^2)$ is the probability density function (PDF) for $N(\mu, \sigma^2)$. The data are two samples taken from the two populations, each of size n, summarized as the sample means: $\boldsymbol{x} = (\bar{x}_1, \bar{x}_2)$. Then

$$B_{10} = \frac{m}{m_0} = \sqrt{\frac{\tau}{\tau + n}} \exp\left\{ \frac{n^2(\bar{x}_1 - \bar{x}_2)^2}{4(\tau + n)} \right\}. \tag{14}$$

Let (μ_{1i}, μ_{2i}), $i = 1, \cdots, N$ be a sample (e.g., an MCMC sample) from the posterior under H_1. Define the *null region* as $\Omega_\epsilon : |\mu_1 - \mu_2| < \epsilon$, a narrow strip in the μ_1–μ_2 plane for H_1 representing the neighborhood of H_0. Then Eq. 10 becomes

$$B_{10} = \lim_{\epsilon \to 0} \frac{\mathbb{P}(\Omega_\epsilon)}{\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})} \approx \frac{\mathbb{P}(|\mu_1 - \mu_2| < \epsilon)}{\mathbb{P}(|\mu_1 - \mu_2| < \epsilon|\boldsymbol{x})}. \quad (15)$$

We estimate $\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})$ by the proportion of posterior samples in which $|\mu_{1i} - \mu_{2i}| < \epsilon$, while the prior probability is $\mathbb{P}(\Omega_\epsilon) \approx 1/\sqrt{4\pi/\tau} \cdot 2\epsilon$. Their ratio (eq. 15) is then an approximation to B_{10} (eq. 14) (see SI text 2).

Example 2. Test of contamination using a Gaussian mixture model

Problem setup. Here we consider a test of contamination under a Gaussian mixture model. This case is not tractable analytically but it yields to numerical calculations. It has most of the irregularities discussed above for the test of introgression, including multiple routes from H_1 to H_0, so we use it to illustrate the application of theorem 1 to calculate B_{10}. We also use this example to study the posterior surface under H_1 under nonstandard conditions.

The data consist of an i.i.d. sample, $\boldsymbol{x} = (x_i)$, with $i = 1, \cdots, n$. The likelihood model specifies $x_i \sim N(0, 1)$ under H_0, or $x_i \sim \alpha N(\mu, 1) + (1 - \alpha)N(0, 1)$ under H_1, with probability α for contamination from $N(\mu, 1)$. Thus H_1 is a mixture of two Gaussian distributions. We assign the prior $p(\alpha, \mu) = \phi(\mu; 0, 1)$ under H_1. There are no nuisance parameters, so the condition on priors (eq. 4) holds.

The marginal likelihoods under the two models are

$$\begin{aligned} m_0 &= \mathrm{e}^{-\frac{1}{2}\sum x_i^2}, \\ m &= \int_0^1 \int_{-\infty}^{\infty} \frac{\mathrm{e}^{-\frac{1}{2}\mu^2}}{\sqrt{2\pi}} \prod_i \left[\alpha\, \mathrm{e}^{-\frac{1}{2}(x_i-\mu)^2} + (1-\alpha)\, \mathrm{e}^{-\frac{1}{2}x_i^2}\right] \mathrm{d}\mu\, \mathrm{d}\alpha, \end{aligned} \quad (16)$$

so that

$$B_{10} = \int_0^1 \int_{-\infty}^{\infty} \frac{\mathrm{e}^{-\frac{1}{2}\mu^2}}{\sqrt{2\pi}} \prod_i \left[1-\alpha+\alpha\, \mathrm{e}^{-\frac{1}{2}(\mu^2-2\mu x_i)}\right] \mathrm{d}\mu\, \mathrm{d}\alpha. \quad (17)$$

We use m_0 to scale the likelihood, and recognise the integrand in eq. 17 as the unnormalized posterior

$$p(\alpha, \mu, \boldsymbol{x}) = \frac{\mathrm{e}^{-\frac{1}{2}\mu^2}}{\sqrt{2\pi}} \prod_i \left[1 - \alpha + \alpha\, \mathrm{e}^{-\frac{1}{2}(\mu^2-2\mu x_i)}\right]. \quad (18)$$

Then the marginal likelihood for H_1

$$M = \int_0^1 \int_{-\infty}^{\infty} p(\alpha, \mu, \boldsymbol{x})\, \mathrm{d}\mu\, \mathrm{d}\alpha. \quad (19)$$

is also the Bayes factor: $M = m/m_0 = B_{10} \equiv B$.

The S-D approach to calculation of the Bayes factor. The constraint that reduces H_1 to H_0 is

$$\begin{aligned} \Omega_0 = &(\alpha = 0, \mu \text{ unidentifiable}) \\ &\cup (\mu = 0, \alpha \text{ unidentifiable}). \end{aligned} \quad (20)$$

The null point comprises two lines or two axes: (a) $\alpha = 0$ and (b) $\mu = 0$ (fig. 2a). Define the null region

$$\Omega_\epsilon = (\alpha < \epsilon_\alpha) \cup (|\mu| < \epsilon_\mu), \quad (21)$$

which are two narrow strips along the axes, of widths ϵ_α and $2\epsilon_\mu$, respectively (fig. 2a). Each strip can be used to calculate the Bayes factor, giving

$$\begin{aligned} B_\alpha(\epsilon_\alpha) &= \frac{\epsilon_\alpha}{\mathbb{P}(\alpha < \epsilon_\alpha \mid \boldsymbol{x})}, \\ B_\mu(\epsilon_\mu) &= \frac{1/\sqrt{2\pi} \times 2\epsilon_\mu}{\mathbb{P}(|\mu| < \epsilon_\mu \mid \boldsymbol{x})}. \end{aligned} \quad (22)$$

We also use both segments to produce a combined estimate, $B_{10} \approx \mathbb{P}(\Omega_\epsilon)/\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})$, with

$$\mathbb{P}(\Omega_\epsilon) = \epsilon_\alpha + \frac{1}{\sqrt{2\pi}} \cdot 2\epsilon_\mu. \quad (23)$$

The shape of the posterior surface. Next we address the question of posterior probabilities for the two regions around the two segments in the parameter space of H_1 when a large dataset is generated under the null model H_0. These represent the proportional contributions of the two null regions to the marginal likelihood (m).

Let $\epsilon_\alpha > 0$ and $\epsilon_\mu > 0$ be fixed small values. We define the probabilities for the two regions as

$$\begin{aligned} P_\alpha &= \mathbb{P}(\alpha < \epsilon_\alpha|\boldsymbol{x}) = \frac{1}{M} \int_0^{\epsilon_\alpha} \int_{-\infty}^{\infty} p(\alpha, \mu, \boldsymbol{x})\, \mathrm{d}\mu\, \mathrm{d}\alpha, \\ P_\mu &= \mathbb{P}(|\mu| < \epsilon_\mu|\boldsymbol{x}) = \frac{1}{M} \int_0^{1} \int_{-\epsilon_\mu}^{\epsilon_\mu} p(\alpha, \mu, \boldsymbol{x})\, \mathrm{d}\mu\, \mathrm{d}\alpha. \end{aligned} \quad (24)$$

When $n \to \infty$, both P_α and P_μ will increase, with $P_\alpha + P_\mu \to 1$. In other words, essentially all posterior density mass will be above the two strips of figure 2a.

We suggest that as $n \to \infty$, P_α does not converge to a point value but instead varies among datasets (all generated under H_0) according to a non-degenerate distribution. Here we provide, without an analytical proof, an intuitive argument through analogy and generate the distribution of P_α numerically for this example.

The problem is similar in nature to the fair-coin or fair-balance paradox, the star-tree paradox (Lewis *et al.*, 2005; Yang and Rannala, 2005; Yang, 2007) and in general comparison of equally correct and nondistinct models (Yang and Zhu, 2018, fig. B2). In the fair-balance paradox, a balance is fair but we test whether it has a negative or positive bias (Yang and Rannala, 2005, eq. 6). We compare two models, $H_- : \mu < 0$ and $H_+ : \mu \geq 0$ when the truth is $\mu = \mu_0 = 0$. The data are an i.i.d. sample of size n, generated from $x_i \sim N(0, 1)$ and summarized as the sample mean $\bar{x}$. With the prior $\mu \sim N(0, 1)$, the posterior model probability is

$$P_- = \mathbb{P}(H_-|\bar{x}) \approx \Phi(-\sqrt{n}\bar{x}), \quad (25)$$

where Φ is the cumulative distribution function (CDF) for $N(0,1)$.

As n is large, $\bar{x}$ is very close to $\mu_0 = 0$ in essentially every dataset. However a small deviation of $\bar{x}$ from 0 can translate to a large departure of P_- from $\frac{1}{2}$. Let $n = 10^6$, say. Then in 2.5% of datasets $\bar{x} < -0.00196$ so that $P_- > 0.975$, while in another 2.5% of datasets $\bar{x} > 0.00196$ so that $P_- < 0.025$. Indeed $\bar{x}$ varies among datasets according to $\bar{x} \sim N\big(0, \frac{1}{n}\big)$, and P_- does not converge to a point but rather behaves like a random number, with the $U(0,1)$ distribution.

Note that a Bayesian test of $H_0 : N(0,1)$ against $H_1 : N(\mu,1)$ with the prior $\mu \sim N(0,1)$ produces

$$B_{10} = \tfrac{1}{\sqrt{1+n}} \exp\Big\{\tfrac{1}{2} n\bar{x}^2 \cdot \tfrac{n}{1+n}\Big\}. \tag{26}$$

As $n\bar{x}^2 \sim \chi^2_1$, $B_{10} \to 0$ when $n \to \infty$. For example, at $n = 10^6$ and $\sqrt{n}|\bar{x}| = 1.96$, we have $B_{10} = 0.00682$, strongly supporting H_0. However, $P_- = \mathbb{P}(\mu < 0|\bar{x}, H_1)$ fluctuates among datasets like a random number.

In the Gaussian-mixture model of figure 2, when n is large enough the posterior probability outside the two strips (represented by Ω_ϵ) will be ~ 0 with $P_\alpha + P_\mu \approx 1$. The two strips are like the parameter space for the two models H_- and H_+ in the fair-balance example, with MLEs under H_1 close to the α and μ axes corresponding to $\bar{x} < 0$ or $\bar{x} > 0$. Thus the posterior surface under H_1 may be expected to vary among replicate datasets (all simulated under H_0) in terms of the probability mass above the two strips. Figure 2d&e shows the unnormalized posterior for two datasets, each of size $n = 10^6$ simulated under H_0. In the first, the MLE is close to the α-axis, while in the second, the MLE is close to the μ-axis. Note that in all large datasets, the MLEs are close to the two axes, supporting the null model of no contamination (fig. 2b).

We simulated 10^3 datasets, each of size $n = 10^6$, under $H_0 : N(0,1)$, and calculated P_α, P_μ of eq. 24 numerically using Gaussian quadrature. The histogram for the rescaled probability, $P'_\alpha = P_\alpha/(P_\alpha + P_\mu)$, is shown in figure 2c.

Example 3. Another Gaussian-mixture model

Problem setup

We alter the previous example slightly, so that H_0 specifies one population with mean μ while H_1 specifies two populations with means μ_1 and μ_2 in proportions α and $1-\alpha$. This example has all the irregularities encountered in the test of unidirectional introgression of figure 1a&b and indeed the two problems have the same geometrical structure (cf: fig. 1d & fig. 3a). This example is not analytically tractable but the MCMC algorithm involves much less computation than the test of introgression (cf: fig. 1f–g & fig. 3b–d).

The parameter vector is $\boldsymbol{\theta}_0 = (\mu)$ for H_0 and $\boldsymbol{\theta} = (\alpha, \mu_1, \mu_2)$ for H_1 (fig. 3a). This is again a test for mixture. The data are an i.i.d. sample, $\boldsymbol{x} = (x_i)$, with $x_i \sim N(\mu,1)$ under H_0, or $x_i \sim \alpha N(\mu_1,1) + (1-\alpha)N(\mu_2,1)$ under H_1. The prior under H_0 is $p_0(\mu) = \phi(\mu; 0, 1/\tau)$, with $\tau = 1$. The prior under H_1 is

$$p(\alpha, \mu_1, \mu_2) = \frac{1}{2\pi/\tau} \exp\Big\{-\frac{\tau}{2}\big(\mu_1^2 + \mu_2^2\big)\Big\}. \tag{27}$$

In other words, $\alpha \sim U(0,1)$ while each of μ_1 and μ_2 is $N(0, 1/\tau)$. If we re-parametrize H_1 and use $\boldsymbol{\theta}' = (\alpha, \mu_1, \delta)$ instead, with $\delta = \mu_2 - \mu_1$, the prior becomes $p(\alpha, \mu_1, \delta) = \phi(\mu_1; -\frac{\delta}{2}, 1/(2\tau))\phi(\delta; 0, 2/\tau)$.

Under H_1, the probability density for each datum x is

$$p(x; \boldsymbol{\theta}) = \tfrac{1}{\sqrt{2\pi}}\Big[\alpha\, \mathrm{e}^{-\frac{1}{2}(x-\mu_1)^2} + (1-\alpha)\, \mathrm{e}^{-\frac{1}{2}(x-\mu_2)^2}\Big], \tag{28}$$

with the posterior

$$p(\boldsymbol{\theta} \mid \boldsymbol{x}) \propto \mathrm{e}^{-\frac{1}{2}(\mu_1^2+\mu_2^2)} \prod_i p(x_i; \boldsymbol{\theta}). \tag{29}$$

There are three routes from H_1 to H_0, and the constraints that reduce H_1 to H_0 are

$$\begin{aligned} \Omega_0 = {} & (\alpha = 0, \mu_1 \text{ unidentifiable}) \\ & \cup (\mu_1 = \mu_2, \alpha \text{ unidentifiable}) \\ & \cup (\alpha = 1, \mu_2 \text{ unidentifiable}). \end{aligned} \tag{30}$$

The null point corresponds to three lines or line segments in the parameter space of H_1, while the null space (the parameter space for H_0) corresponds to three planes in the parameter space of H_1 (fig. 3a).

We note that the condition on priors (eq. 4) holds for segments (a) $\alpha = 0$ and (c) $\alpha = 1$, but not for segment (b) $\mu_1 = \mu_2$ or $\delta = \mu_2 - \mu_1 = 0$. For segment (b), we have $p_0(\mu) = \phi(\mu; 0, 1/\tau)$ for H_0, and $p(\mu_1 \mid \delta_0) = \phi(\mu_1; 0, 1/(2\tau))$ for H_1, so there is a mis-match.

Bayes factor via the S-D density ratio

We use the differentials ϵ_α and $\epsilon_{\mu_1} = \epsilon_{\mu_2} = \epsilon_\mu$ to define a null region in the parameter space of H_1,

$$\begin{aligned} \Omega_\epsilon &= \{\Omega_{\epsilon,a} \cup \Omega_{\epsilon,b} \cup \Omega_{\epsilon,c}\} \\ &= \{\alpha < \epsilon_\alpha \cup |\mu_2 - \mu_1| < \epsilon_\mu \cup 1 - \alpha < \epsilon_\alpha\}. \end{aligned} \tag{31}$$

If we use each segment to calculate B_{10}, we have

$$\begin{aligned} B_a &= \frac{\mathbb{P}(\Omega_{\epsilon,a})}{\mathbb{P}(\Omega_{\epsilon,a} \mid \boldsymbol{x})} = \frac{\epsilon_\alpha}{\mathbb{P}(\alpha < \epsilon_\alpha \mid \boldsymbol{x})}, \\ B_b &= \frac{\mathbb{P}(\Omega_{\epsilon,b})}{\mathbb{P}(\Omega_{\epsilon,b}|\boldsymbol{x})} = \frac{1/\sqrt{2\pi/\tau} \times 2\epsilon_\mu}{\mathbb{P}(|\mu_1 - \mu_2| < \epsilon_\mu \mid \boldsymbol{x})}, \\ B_c &= \frac{\mathbb{P}(\Omega_{\epsilon,c})}{\mathbb{P}(\Omega_{\epsilon,c} \mid \boldsymbol{x})} = \frac{\epsilon_\alpha}{\mathbb{P}(1 - \alpha < \epsilon_\alpha \mid \boldsymbol{x})}. \end{aligned} \tag{32}$$

We use the MCMC sample of figure 3d to calculate the D-S density ratio with different differentials ϵ_α and ϵ_μ (fig. S2b). We expect B_a and B_c to approximate B_{10} but B_b may be off by a factor, since the condition on priors holds for segments a and c but not for segment b. However, for this dataset the three segments produced

similar B_{10} values, around 0.25, with the data preferring the null model of no mixture (i.e., the true model). Note that under H_1, $\boldsymbol{\theta} = (\alpha, \mu_1, \mu_2)$ and $\boldsymbol{\theta}' = (1-\alpha, \mu_2, \mu_1)$ are unidentifiable and this is an unidentifiability of the label-switching type. The prior and posterior around segments a and c are mirror images of each other, and B_a and B_c differ only because the MCMC sample is finite.

If we use all segments to calculate a combined S-D density ratio, the prior probability for the null region under H_1 is

$$\begin{aligned}\mathbb{P}(\Omega_\epsilon) &= \mathbb{P}\{\alpha < \epsilon_\alpha \cup |\mu_2 - \mu_1| < \epsilon_\mu \cup 1-\alpha < \epsilon_\alpha\} \\ &\approx p(\alpha = 0)\epsilon_\alpha + p(\delta = 0)\cdot 2\epsilon_\mu + p(\alpha = 1)\epsilon_\alpha,\end{aligned} \tag{33}$$

while the posterior probability can be calculated by processing the MCMC sample under H_1,

$$\begin{aligned}\mathbb{P}(\Omega_\epsilon|\boldsymbol{x}) &= \mathbb{P}\left\{\alpha < \epsilon_\alpha \cup |\mu_2 - \mu_1| < \epsilon_\mu \cup 1-\alpha < \epsilon_\alpha \,\middle|\, \boldsymbol{x}\right\} \\ &\approx p(\alpha = 0|\boldsymbol{x})\epsilon_\alpha + p(\delta = 0|\boldsymbol{x})\cdot 2\epsilon_\mu \\ &\quad + p(\alpha = 1|\boldsymbol{x})\epsilon_\alpha.\end{aligned} \tag{34}$$

In the example of figure 3d, the different segments produced similar B_{10} estimates (fig. S2b), so that the combined estimate is similar as well.

Test of unidirectional introgression (UDI) between sister lineages

The test of introgression (under the UDI model) has been introduced in Introduction (fig. 1). The null model H_0 assumes no gene flow, with the parameter vector $\boldsymbol{\theta}_0 = (\tau, \theta)$, while the alternative model H_1 assumes unidirectional introgression, with the parameter vector $\boldsymbol{\theta} = (\varphi, \tau_Y, \tau_R, \theta)$ (fig. 1b). Here we assume the same population size θ for all species on the phylogeny, which has the same prior under H_0 and H_1. In models with different population sizes, the θ parameters have the same priors in the two models as well. There are three routes from H_1 to H_0, and the null point (one point in the parameter space of H_0) corresponds to three lines or line segments in the parameter space of H_1 given by the constraints

$$\Omega_0 = \{\varphi = 0\} \cup \{\tau_Y = \tau_R\} \cup \{\varphi = 1\} \tag{35}$$

(table 1(i), fig. 1d). The null space (the parameter space for H_0) is represented by the three planes in figure 1d.

In BPP, we assign a beta prior on $\varphi \sim \text{beta}(1,1)$, a gamma-Dirichlet prior on the divergence times (Yang and Rannala, 2010, eq. 2), which means that $\tau_R \sim G(\alpha_\tau, \beta_\tau)$, and given τ_R, $\beta = \tau_Y/\tau_R$ is $U(0,1)$. We note that the condition on priors (eq. 4) holds for segments (a) and (b), but not for segment (c) (table 1(i)). For segments (a) and (b), the nuisance parameters are τ_R and θ), each with the same gamma prior in both H_0 and H_1. For segment c, the nuisance parameter τ_Y in H_1 is mapped onto τ in H_0, with different priors. In H_0 the prior is $\tau \sim G(\alpha, \beta)$, with the prior mean α/β. In H_1, we have $\tau_Y|\tau_R \sim U(0,1)$ with $\tau_R \sim G(\alpha, \beta)$ so that

$$\begin{aligned}p(\tau_Y) &= \int_0^\infty \frac{1}{\tau_R}\cdot\frac{\beta^\alpha}{\Gamma(\alpha)}\tau_R^{\alpha-1}\,\mathrm{e}^{-\beta\tau_R}\,\mathrm{d}\tau_R \\ &= \tfrac{\beta}{\alpha-1}\left[1 - G(\tau_Y, \alpha-1, \beta)\right],\end{aligned} \tag{36}$$

where $G(y, \alpha, \beta)$ is the CDF for the gamma distribution $G(\alpha, \beta)$ (Flouri *et al.*, 2022). This has the prior mean $\alpha/(2\beta)$.

From eq. 35, we define the null region

$$\Omega_\epsilon = \{\varphi < \epsilon_\varphi\} \cup \{1-\beta < \epsilon_\beta\} \cup \{1-\varphi < \epsilon_\varphi\} \tag{37}$$

to calculate the Bayes factor, $B_{10} \approx \frac{\mathbb{P}(\Omega_\epsilon)}{\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})}$, where $\mathbb{P}(\Omega_\epsilon) = \epsilon_\varphi + \epsilon_\beta + \epsilon_\varphi$ while $\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})$ is the proportion of MCMC samples under the UDI model that fall in the null region (Ω_ϵ).

We use this approach in our simulation below, setting the differentials to be 0.01σ where σ is the prior standard deviation; in other words we use $\epsilon_\varphi = \epsilon_\beta = 0.01\frac{1}{\sqrt{12}} = 0.002886$.

Test of bidirectional introgression (BDI) between sister lineages

Under the BDI model there are two introgression probabilities, so that the parameter vector in H_1 is $\boldsymbol{\theta} = (\varphi_X, \varphi_Y, \tau_Y, \tau_R, \theta)$. The null model H_0 is the MSC model with no gene flow as before, with $\boldsymbol{\theta}_0 = (\tau, \theta)$ for H_0. The common population size parameter θ is a nuisance parameter that exists in both models, with the same prior.

There are five routes by which H_1 can be reduced to H_0. In other words, one point in H_0 (the null point) corresponds to five lines or regions specified by equality constraints on four parameters in H_1: φ_X, φ_Y, τ_Y, and τ_R (table 1(ii)),

$$\begin{aligned}\Omega_0 = &\{\varphi_X = 0, \varphi_Y = 0\} \\ &\cup \{\varphi_X = 1, \varphi_Y = 1\} \\ &\cup \{\tau_Y = \tau_R\} \\ &\cup \{\varphi_X = 0, \varphi_Y = 1\} \\ &\cup \{\varphi_X = 1, \varphi_Y = 0\}.\end{aligned} \tag{38}$$

The condition on priors (eq. 4) holds for segments (a)–(c) but not for segments (d) and (e) (table 1(ii)). For the latter two, the nuisance parameter τ_Y in H_1, which has the prior of eq. 36, is mapped onto τ in H_0, with the prior $\tau \sim G(\alpha, \beta)$, so there is a mismatch.

From eq. 38, define the null region as

$$\begin{aligned}\Omega_\epsilon = &\{\varphi_X < \epsilon_\varphi, \varphi_Y < \epsilon_\varphi\} \\ &\cup \{1-\varphi_X < \epsilon_\varphi, 1-\varphi_Y < \epsilon_\varphi\} \\ &\cup \{1-\beta < \epsilon_\beta\} \\ &\cup \{\varphi_X < \epsilon_\varphi, 1-\varphi_Y < \epsilon_\varphi\} \\ &\cup \{1-\varphi_X < \epsilon_\varphi, \varphi_Y < \epsilon_\varphi\}.\end{aligned} \tag{39}$$

As in the test of UDI, we set $\epsilon_\varphi = \epsilon_\beta = 0.002886$ to process the MCMC sample from the BDI model to calculate $\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})$. For the prior probability, we have

$$\mathbb{P}(\Omega_\epsilon) = \epsilon_\varphi^2 + \epsilon_\varphi^2 + \epsilon_\beta + \epsilon_\varphi^2 + \epsilon_\varphi^2 \tag{40}$$

We use this approach in the computer simulation below.

Posterior probabilities for different regions of the parameter space in H_1. We expect our theory for posterior probabilities for different regions of the parameter space in H_1 when a large dataset is generated under the null model discussed in Example 2 applies to the UDI and BDI models. If the data are generated under the MSC model with no gene flow, and analyzed under the UDI model, the MCMC will visit the three regions of the parameter space in the neighborhood of the three segments (fig. 1d, table 1(i)) with different probabilities among datasets. There will be no convergence to a point. In some datasets, the chain may spend more time close to the red line (segment a), while in others (all generated under H_0), the chain may spend more time close to the gree line (segment b), and so on. The biological scenario of no gene flow is represented by $\varphi = 0$, or 1, or any value over (0, 1).

Similarly if large datasets are generated under the MSC model of no gene flow and analyzed under the BDI model, the MCMC will visit the five regions of the parameter space in the neighborhood of the five segments (table 1(ii)).

Materials and Methods

Simulation under the null model of no gene flow

To verify our theory for applying the S-D approach to calculating the Bayes factor and to examine the false-positive rate of the Bayesian test of gene flow, we conducted a small simulation under the null model of MSC with no gene flow (fig. 1a). We used the population size $\theta = 0.005$ and three values for the species split time: $\tau_R = \frac{1}{2}\theta$, θ, and 10θ, representing very shallow, shallow, and deep divergences, respectively. Both θ and τ are measured by the number of mutations per site. With this mutational time scale, the average coalescent time between two sequences sampled from a population of size θ is $\theta/2$, so that $\tau = \theta/2$ represents a very shallow tree. Each dataset consisted of $L = 500$ loci, with 20 haploid sequences per locus (10 per species), and with the sequence length to be 500. The number of replicates was 100. The `simulate` option of BPP was used to generate gene trees for the loci and then to 'evolve' sequences along branches of the gene trees under the JC model (Jukes and Cantor, 1969) to generate sequence alignments.

Each dataset was analyzed using BPP under the MSC-I (UDI and DBI) and MSC-M models of figure 4b–d to conduct the Bayesian test. In analyses under the MSC-I models, the age of the root τ_R was assigned a gamma prior $G(2, 400)$, with the prior mean 0.005, too large for data simulated using $\tau_R = \frac{1}{2}\theta$ and too small for $\tau_R = 10\theta$. Given τ_R, τ_X under UDI and BDI has the uniform distribution. The option `thetamodel = linked-all` in BPP was used so that the same population size (θ) was assumed for all populations on the species tree, assigned the gamma prior $\theta \sim G(2, 400)$ with mean 0.005. Note that models with different θs for species on the phylogeny are available in BPP, but in this paper assumptions on θs are unimportant. The introgression probability φ (or both φ_Y and φ_X in the BDI model) was assigned the prior beta(1, 1).

We used a burn-in of 40,000 iterations, after which we took 10^5 samples, sampling every 2 iterations. Running time for analyzing one dataset was ~ 11 hours using one thread.

The MCMC sample under H_1: MSC-I was processed to calculate the Bayes factor via the S-D density ratio. For test of H_0: MSC against H_1: MSC-I (UDI), we used 1% of the prior standard deviations as the differentials, so that $\epsilon_\varphi = \epsilon_\beta = 0.01/\sqrt{12} = 0.002886$. From eq. 35, the null region is

$$\Omega_\epsilon = (\varphi < \epsilon_\varphi) \cup (1 - \beta < \epsilon_\beta) \cup (1 - \varphi < \epsilon_\varphi),$$

where $\beta = \tau_Y/\tau_R$ (eq. 35). We calculate the Bayes factor as $B_{10} \approx \frac{\mathbb{P}(\Omega_\epsilon)}{\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})} = \frac{3\epsilon}{\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})}$.

We also tested the idea of using segments a and b only, for which the condition on priors holds (eq. 4), with

$$\Omega_\epsilon = (\varphi < \epsilon_\varphi) \cup (1 - \beta < \epsilon_\beta),$$

and $B_{10} \approx \frac{2\epsilon}{\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})}$. This produced nearly identical results as the use of all three segments.

For testing BDI, we used the null region defined in eq. 38. As in the test of UDI, we set $\epsilon_\varphi = \epsilon_\beta = 0.002886$. We have (see eq. 40)

$$B_{10} \approx \frac{\mathbb{P}(\Omega_\epsilon)}{\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})} = \frac{\epsilon_\varphi^2 + \epsilon_\varphi^2 + \epsilon_\beta + \epsilon_\varphi^2 + \epsilon_\varphi^2}{\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})}.$$

If we use only segments a, b, c, for which the condition on priors hold (table 1(i)), we have

$$\begin{aligned}\Omega_\epsilon = &\{\varphi_X < \epsilon_\varphi, \varphi_Y < \epsilon_\varphi\}\\ &\cup \{1 - \varphi_X < \epsilon_\varphi, 1 - \varphi_Y < \epsilon_\varphi\}\\ &\cup \{1 - \beta < \epsilon_\beta\},\end{aligned}$$

and $B_{10} \approx \frac{\epsilon_\varphi^2 + \epsilon_\varphi^2 + \epsilon_\beta}{\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})}$. This produced nearly identical results as the use of all five segments.

The same data, simulated under H_0, were also analyzed under the migration model (H_1: MSC-M, fig. 4c) to conduct the Bayesian test. The MSC-M model assumes that migration from $A \to B$ occurs continuously in every generation (Flouri *et al.*, 2023). Version 4.8 of BPP uses the mutational time scale to define migration rates. The mutation-scaled migration rate is defined as $\varpi_{XY} = \frac{m_{XY}}{\mu} = \frac{4M_{XY}}{\theta_Y}$, where m_{XY} is the proportion of immigrants in the recipient population Y from population X, $M_{XY} = m_{XY}N_Y$ is the expected number of $X \to Y$ immigrants per generation, and μ is neutral mutation rate per site per generation. We used two priors to assess the impact of the prior, $\varpi_{XY} \sim G(2, 0.01)$ with mean 200 and $G(2, 1)$ with mean 2. Like the analysis under the MSC-I models (UDI and BDI), we assumed the same population size for all species (`thetamodel = linked-all`). The S-D approach was used to calculate the Bayes factor B_{10}, using the null region Ω_ϵ: $\varpi < \epsilon_\varpi = 0.005\sigma, 0.01\sigma, 0.05\sigma$, where σ is the prior

standard deviation. Then $B_{10} \approx \mathbb{P}(\Omega_\epsilon)/\mathbb{P}(\Omega_\epsilon|\boldsymbol{x})$, with $\mathbb{P}(\varpi < \epsilon_\varpi)$ given by the CDF for the gamma prior.

Simulation under models of gene flow

To assess the power of the Bayesian test of gene flow between sister lineages and the precision of Bayesian estimation of the rate of gene flow, we simulated data under the MSC-I and MSC-M models of figure 4b&c, with $A \to B$ (or $X \to Y$) gene flow. Most settings were the same as for the simulation under the null model. As before we used three species split times: $\tau_R = \frac{1}{2}\theta$, θ, and 10θ, with $\theta = 0.005$. Under the MSC-I model, the introgression time was set at $\tau_Y = \frac{1}{4}\theta$, while the introgression probability varied: $\varphi = 0.05, 0.1, 0.2, 0.3, 0.5, 0.7$. For the MSC-M model, the population migration rate (from $A \to B$) was $M = 0.05, 0.1, 0.2, 0.3, 0.5, 1$. Each dataset consists of $L = 500$ loci, with $10 + 10$ sequences, with the sequence length 500. The number of replicates is 100.

Each replicate dataset was analyzed under the three models of gene flow of figure 4b–d: MSC-I (UDI), MSC-I (BDI), and MSC-M. One population size (θ) was assumed for all species. The priors and MCMC settings were as above for analysis of data simulated under the null model. The Bayesian test of gene flow was conducted as before, using the S-D density ratio to calculate the Bayes factor.

Bayesian inference of gene flow between two lizard species

We analyze a dataset of 1000 ddRAD loci from three Baja California lizard species, *Sceloporus orcutti* (O), *S. hunsakeri* (H), and *S. licki* (L), published and analyzed by Gottscho *et al.* (2025). There are 16, 16, and 12 haploid sequences per locus for O, H, and L, respectively, with the sequence length to be ~90bps. The species phylogeny is shown in figure 5a, with possible gene flow between *S. orcutti* and *S. hunsakeri*.

We assign the gamma prior $\tau_r \sim G(2, 200)$ with mean 0.01 on the age of the root, $\theta \sim G(2, 100)$ with mean 0.005 for the population sizes, and the uniform prior $U(0, 1)$ on the introgression probabilities $(\varphi_{oh}, \varphi_{ho})$. For the MCMC, we used a burn-in of 40,000 iterations, after which we took 2×10^5 samples, sampling every 2 iterations. The run took 11hrs using 36 threads on a server. The MCMC samples were processed to calculate the Bayes factor for testing the hypothesis of no gene flow using the S-D density ratio, as described in the paper.

The same data were also analyzed under the MSC-M model with bidirectional migration. The migration rates $(\varpi_{oh}, \varpi_{ho})$ were assigned the gamma prior $G(2, 0.1)$ with mean 20. Other settings were the same as for the BDI model. Running time was 15hrs using 36 threads.

Results

Bayesian test of gene flow between sister species has low false positives

Results for the Bayesian test of gene flow using data simulated under the null model (MSC, fig. 4a) are summarized in table 2. We discuss the results with $\epsilon_\varphi, \epsilon_\beta$ in MSC-I and ϵ_ϖ in MSC-M set to 0.01σ, where σ is the prior standard deviation, but note that use of 0.005σ and 0.05σ produces very similar results.

The Bayes factor B_{10} is calibrated with reference to posterior model probabilities $\mathbb{P}(H_1|\boldsymbol{x})$. With equal prior probabilities for the two models ($\frac{1}{2}$), $\mathbb{P}(H_1|\boldsymbol{x}) = 0.01$, 0.05, 0.95, and 0.99 correspond to $B_{10} = 0.0101, 0.0526$, 19, and 99, respectively. At the 5% level (or $B_{10} > 19$), the false positive rate of the Bayesian test is 0 for all but two settings, where the rate is 1% and 2%, lower than the nominal 5% (table 2). Here we are examining a Frequentist property (the type-I error rate) of a Bayesian test, so one does not expect a match between the error rate and the significance level. Nevertheless, the false-positive rate of the Bayesian test is noted to be well below the nominal significance level.

Note that the Bayesian test makes a 'stronger' inference than the LRT in that it can strongly reject H_1 and support H_0 whereas the LRT may fail to reject H_0 but will never strongly support it. At the 5% level ($B_{10} < 0.0526$), H_1 is rejected in 72% and 100% of datasets simulated at deep divergence ($\tau = 10\theta$) when the alternative hypothesis is MSC-I UDI and MSC-M, respectively (table 2). Strong support for H_0 rarely occurs at shallow divergence ($\tau = \frac{1}{2}\theta$ or θ), suggesting that the data are far more informative concerning gene flow at deep divergence.

Posterior means and the 95% highest posterior density (HPD) credible intervals (CIs) for parameters in the three alternative models of gene flow (UDI, BDI, and MSC-M; fig. 4b–d) are summarized in figure S3. We first consider the UDI model. The population size parameter θ is well estimated, but the other parameters $(\tau_R, \tau_Y, \varphi_Y)$ are not. In particular the introgression probability φ_Y has CIs that cover nearly the whole range $(0, 1)$. However, the summaries in figure S3 do not accommodate the fact that all of the three segments (a), (b), and (c) of figure 1d represent the null model of no gene flow. For example, both $\varphi = 0$ and $\varphi = 1$, as well as any value for φ (when $\tau_Y = \tau_R$), represent the true model of no gene flow and are thus plausible, but the posterior mean and the HPD CI of φ calculated using the MCMC sample are implausible and unsupported by data. Note that even though the posterior for φ_Y covers the whole range (0–1), it is incorrect to conclude that the data are uninformative about the presence or absence of gene flow. As mentioned above, the model of gene flow is rejected in many datasets.

Similarly under the BDI model the neighborhoods of the five segments/regions that represent the null model of no gene flow (table 1(ii)) represent the same biological scenario of no gene flow, and this fact is not accommodated in the summaries for φ_X and φ_Y in figure S3.

The MSC-M model does not involve the irregularities of the MSC-I models discussed in this paper. Posterior summaries of parameters under the model (θ, τ_R, M) are all well-behaved and easy to interpret (fig. S3). In particular, estimates of M are close to 0 (the true value), supporting the null model of no gene flow. The data are far more informative (with better estimates of M) on the deep tree than on the shallow trees.

Bayesian test of gene flow between sister species has high power

Results of the Bayesian test when data were simulated under the MSC-I and MSC-M models of figure 4b&c, with $A \to B$ (or $X \to Y$) gene flow are summarized in figure 6. First we consider data simulated under UDI (fig. 6a–c). When the data are analyzed under the correct UDI model (I-UDI), the test has ~100% power except for the very shallow tree ($\tau_R = \frac{1}{2}\theta$) and low rates of gene flow ($\varphi \le 0.1$). For data simulated under UDI, the BDI model is over-parametrized but not misspecified. The test under the BDI showed similar performance to the use of the UDI model. We implemented the MSC-M model under two priors, $\varpi \sim G(2, 0.01)$ with mean 200 and $\varpi \sim G(2, 1)$ with mean 2. The test using the MSC-M model, in particular, under the $\varpi \sim G(2, 1)$ prior (fig. 6c, I-M) had lower power than under UDI or BDI. In particular, power dropped when the introgression probability is very high. We note that when $\varphi > \frac{1}{2}$, most sequences from B are traced to the introgression parent A. It should be noted that in all datasets and all models, $B_{10} > 1$ (fig. 6a–c), suggesting that the alternative hypothesis of gene flow is supported in the Bayesian test, even if the support may not reach the cutoff of $B_{10} > 19$ or 99.

Next we consider data simulated under the MSC-M model (fig. 6d–f). The test has full power at the 1% level (with $B_{10} > 99$) under the two MSC-I models (M-UDI, M-BDI) for all three species divergence times and for all levels of migration rates. Misspecification of the mode of gene flow (i.e., the true model assumes continuous gene flow while the analysis model is discrete) has not had any major impact. The test under MSC-M had full power except for the combination of $M = 1$ and the $\varpi \sim G(2, 1)$ prior. We note that just as $\varphi \approx 1$ in MSC-I represents the scenario of isolation with no gene flow, $M = \infty$ represents the scenario of panmixia, with no gene flow.

We note that overall there is a greater amount of gene flow in data simulated under the MSC-M model. The expected amount of introgression under the MSC-M model, measured by the probability that any sequence from species B is traced to population A (irrespective of the migration time), when one traces the genealogical history of the sampled sequences backward in time, is

$$\varphi_0 = 1 - e^{-\frac{4M_{AB}}{\theta_B}\tau_R} \tag{41}$$

(Huang *et al.*, 2022, eq. 10). At the low rate of $M = 0.05$, $\varphi_0 = 0.095, 0.181, 0.865$ for the three divergence levels (fig. S4), higher than the low rate $\varphi = 0.05$ in the MSC-I (UDI) model. This should explain why all tests have full power at the low rate in data simulated under MSC-M. At the high rate of $M = 1$, we have $\varphi_0 = 0.865, 0.982, 1.000$, higher than $\varphi = 0.7$ in the UDI model. Data simulated under the MSC-M model with $\varphi_0 \approx 1$ may be expected to resemble data simulated under UDI with $\varphi = 1$ (which is equivalent to the MSC model of no gene flow; see fig. 1c). This may explain the loss of power of the Bayesian test to detect gene flow at very high migration rates (M).

Compared with the true values used in the simulation, $\varpi = 40, 80, 160, 240, 400$ and 800 at the six values of M, the prior $\varpi \sim G(2, 1)$ has a mean that is far too small, creating conflicts between the prior and the data and leading to reduced power (fig. 6d–f′). In practical data analysis, it seems that $G(2, 0.1)$ with the prior mean 20 may be a good choice.

Bayesian estimation of the rate of introgression between sister species is reliable only at deep species divergences

We examine the precision and accuracy of parameter estimation in data analyzed in figure 6, simulated under models with gene flow. In particular, we are interested in the rate of gene flow (φ under MSC-I and M under MSC-M; fig. 4b–d). The results are summarized in figure 7.

First we consider data simulated under the UDI model (fig. 7a–c). Under the correct UDI model, species split time τ_R and the population size (θ) are well estimated. Note that we assumed the same population size for all species on the tree, so there is a huge amount of information in the data about θ. The introgression time τ_Y and the introgression probability φ involved large uncertainties when the true φ is small (< 0.2, say). When φ is very small, the null model of no gene flow is nearly correct, and the three segments of figure 1c (where $\varphi = 0, 1$, or any value between 0–1) are expected to fit the data nearly equally well; in other words, there exist ridges in the posterior surface close to the three segments of figure 1c, and the data are not informative about φ. The poor estimation of τ_Y and φ form a sharp contrast with the precise and accurate estimation of τ_R and θ. Furthermore, the species split time has a large impact, with the CIs for φ to be much narrower for the deep tree (with $\tau_R = 10\theta$) than for the very shallow tree (with $\tau_R = \frac{1}{2}\theta$) (fig. 7).

The analysis under BDI (fig. 7b, I-BDI) produces similar estimates of the species split time and introgression time (τ_R, τ_Y) to those under UDI, but the estimates of the introgression probabilities (φ_X, φ_Y) show interesting patterns, different from the analysis under UDI (the I-UDI setting). For data generated at low φ in the true UDI model, both φ_X and φ_Y are poorly estimated. This is similar to the poor estimation of φ_Y under UDI, due to the issue with the summary: at the low rate, the null model of no gene flow is nearly correct, and the four lines and a plane under the BDI model (eq. 38, table 1(ii)), where φ_X and φ_Y are either 0 or 1 or anywhere over (0, 1), are expected to fit the data nearly equally well. At high φ in the true UDI model, the BDI model

underestimates φ_Y (which has the true value φ) and overestimates φ_X (which has the true value 0), having difficulty in determining the direction of introgression. This may reflect the label-switching unidentifiability problem for the BDI model, as discussed by Yang and Flouri (2022). Under the BDI model, without constraints on θ, two sets of parameter values, $\boldsymbol{\theta} = (\varphi_X, \varphi_Y, \theta_X, \theta_Y)$ and $\boldsymbol{\theta}' = (1-\varphi_X, 1-\varphi_Y, \theta_Y, \theta_X)$, are unidentifiable (Yang and Flouri, 2022, fig. 1). Similar unidentifiability occurs when all populations are assumed to have the same size (θ).

Next we consider data simulated under the MSC-M model (fig. 7d–f). Estimates under UDI and BDI at shallow divergence ($\tau_R = \frac{1}{2}$) and low migration rate (M) are very poor. As discussed above, when the null model of no gene flow is nearly correct, our summaries in figure 7 do not account for the fact that different parts of the parameter space represent the same biological scenario of no gene flow. When the migration rate is higher, the estimates improve, in particular at high divergence. However, when M is very high, the model will again be close to the null model of no gene flow. In general the MSC-I model does not recover all the gene flow that has occurred. The results are similar to previous simulations in which data were generated under the MSC-M model and analyzed under the MSC-I model (Huang *et al.*, 2022, φ against M under the IM model).

In the M-M setting, the MSC-M model with $A \to B$ migration is used to both simulate and analyze the data (fig. 7d–f). This case shows a complex pattern with respect to the species divergence time (τ_R) and the migration rate M. When M is low (0.05 or 0.1), both θ and τ are well estimated, as is the migration rate M. When $0.2 \le M \le 0.5$, the estimates are good for the shallow trees only ($\tau_R = \frac{1}{2} \times \theta$ or θ), but poor for the deep tree. The prior on ϖ affected the posterior estimate (fig. 7f&f′). Note that at $M \ge 0.2$, $\varphi_0 = 1.000$ on the deep tree (fig. S4), and at such extreme amount of gene flow, the estimate of τ_R may collapse.

Asymptotic analysis to assess the impact of species split time

To understand the effect of species divergence time on the information content in the data concerning the introgression probability φ under the UDI model (fig. 4b), we consider the simple case of only two sequences per locus, with one sequence per species. The large-sample behavior of the estimation method (when the number of loci $L \to \infty$) is analytically tractable (Huang *et al.*, 2022). We assume one θ for all populations, so there are 4 parameters in the model, $\boldsymbol{\theta} = (\varphi, \tau_Y, \tau_R, \theta)$. Let $\boldsymbol{\theta}^* = (\varphi^*, \tau_Y^*, \tau_R^*, \theta^*)$ be the true parameter values. The alignment at each locus is summarized as the observed number (x) of differences at n sites. Let the probability of observing x differences at n sites be $p(x; \boldsymbol{\theta})$, given by Huang *et al.* (2022, eq. S9). When the number of loci $L \to \infty$, the per-locus log likelihood becomes

$$\ell(\boldsymbol{\theta}) = \sum_{x=0}^{n} p(x; \boldsymbol{\theta}_*) \log p(x; \boldsymbol{\theta}). \tag{42}$$

Note that here n is the sequence length so that the number of variable sites (x) has the range $(0, n)$.

We fix the population size parameter θ at the true value, as it is typically well estimated. For given τ_Y and φ, we estimate τ_R by maximizing ℓ, and calculate the optimized ℓ as a function of the other two parameters (fig. 8). We used three values for τ_R: $\frac{1}{2}\theta$, θ, and 10θ, and three values for φ: 0.01, 0.1, and 0.6. The log-likelihood contour for τ_Y and φ is shown in figure 8.

When $\varphi = 0.01$ and the true $\tau_R = \frac{1}{2}\theta$, the log-likelihood surface has an elongated foot shape, similar to the Bayesian posterior surface for the two parameters of figure 1f. Information content on φ improved when the species split time is larger ($\tau_R = 10\theta$) or when the rate of gene flow is higher ($\varphi = 0.1$ or 0.6). In particular, as φ increased, the contour lines became more circular, resulting in narrower CIs for both parameters (fig. 8).

In the case of two sequences per locus and treating the coalescent time as data, Thawornwattana *et al.* (2023, eq. 2) give the Fisher information for estimating φ_Y as

$$I \approx \frac{P_X}{\varphi_Y(1-\varphi_Y P_X)}, \tag{43}$$

where $P_X = 1 - \mathrm{e}^{-\frac{2}{\theta_X}(\tau_R - \tau_Y)}$ is the probability that two sequences (a, b) entering population X coalesce in population X rather than in population R (fig. 4b). The data are more informative about φ_Y if P_X if larger or if the species divergence is deeper, with $\tau_R \gg \tau_Y$.

Inference of gene flow between Sceloporus *lizard species*

We analyze a ddRAD dataset from three *Sceloporus* lizard species, *Sceloporus orcutti* (O), *S. hunsakeri* (H), and *S. licki* (L) to test for gene flow between the sister species *S. orcutti* and *S. hunsakeri* (fig. 5a). We run BPP under the bidirectional introgression (BDI) and migration (BDM) models. First we note that estimates of parameters shared between the two models are very similar (table 3). The introgression time under BDI model is nearly zero, indicating that gene flow between the two species stopped very recently or may be ongoing. The rate of gene flow is small, with $\varphi_{oh} = 0.0044$ (with the 95% HPD CI 0.0013–0.0079) and $\varphi_{ho} = 0.0022$ (0.0008–0.0038) (fig. 5b, table 3). The BDM model assumes ongoing gene flow, with $M_{oh} = M_{ho} = 0.0033$ migrant individuals in each direction per generation (table 3). Those rates translate to $\varphi_{0,o\to h} = 1 - \mathrm{e}^{-4*0.003433/0.003388\times 0.007606} = 0.0304$, and $\varphi_{0,h\to o} = 0.0125$ according to eq. 41, suggesting that the migration model recovers more gene flow than the MSC-I model. Overall the posterior estimates suggest a small but significant amount of gene flow in each direction, and gene flow may be ongoing or stopped a short time ago.

We then applied the Bayesian test. Under the BDI model, one can test $H_0 : \varphi_{oh} = 0, \varphi_{ho} = 0$ against $H_1 : \varphi_{oh} \neq 0, \varphi_{ho} \neq 0$ (table 1(ii)). Rejection of H_0 means introgression in at least one direction. We can use each of the five segments for which the condition on priors

holds to calculate the Bayes factor. All calculations lead to strong support for gene flow (with $B \approx \infty$).

Figure 5d illustrates the test of $H_0 : \varphi_{oh} = 0$ against $H_1 : \varphi_{oh} \neq 0$, with φ_{ho} treated a nuisance parameter (present in both H_0 and H_1). There exist three segments, each of which can be used to calculate B_{10}, as described in table 1(i). Here we use the first segment, with $B_{10} \approx \frac{\mathbb{P}(\varphi_{ho}<\epsilon)}{\mathbb{P}(\varphi_{ho}<\epsilon|\boldsymbol{x})}$. The data support $h \to o$ introgression, but the introgression probability is very low. However, the low introgression probabilities cause some challenges to applying the S-D approach.

The test under the BDM model does not involve the irregularities of the UDI or BDI models. In figure 5e, we use the same MCMC sample under BDM for figure 5c to calculate the Bayes factor for testing three null hypotheses. The first is $H_0 : \varpi_{oh} = 0$, against $H_1 : \varpi_{oh} > 0$, with ϖ_{ho} treated as a nuisance parameter. We have $B_{oh} \approx \frac{\mathbb{P}(\varpi_{oh}<\epsilon)}{\mathbb{P}(\varpi_{oh}<\epsilon|\boldsymbol{x})}$ where $\mathbb{P}(\varpi_{oh} < \epsilon|\boldsymbol{x})$ is estimated by the proportion of MCMC samples in which $\varpi_{oh} < \epsilon$. Note that with the gamma prior, the tail probability $\alpha = \mathbb{P}(\varpi_{oh} < \epsilon) = G(\epsilon; 2, 0.1) = 0.001, 0.005$, gives the small value $\epsilon = 0.454, 1.035$, where $G(\epsilon; 2, 0.1)$ is the CDF of the gamma prior. In the second test we have $H_0 : \varpi_{ho} = 0$ against $H_1 : \varpi_{ho} > 0$. The third test is for $H_0 : \varpi_{oh} = 0, \varpi_{ho} = 0$ against $H_1 : \varpi_{oh} > 0, \varpi_{ho} > 0$. We have $B_2 \approx \frac{\mathbb{P}(\varnothing)}{\mathbb{P}(\varnothing|\boldsymbol{x})}$, calculated by defining $\varnothing = \{\varpi_{oh} < \epsilon, \varpi_{ho} < \epsilon\}$. The three Bayes factors (B_{oh}, B_{ho}, B_2) are plotted against the tail probability α in figure 5e. All of them are $\gg 1$, strongly supporting gene flow although there is a lack of precision when B is very large.

Discussion

Bayesian testing of gene flow between sister lineages and calculation of the Bayes factor

Considered a problem of statistical hypothesis testing (Jeffreys, 1935), the test of introgression between sister lineages involves a whole suite of challenging nonstandard features, including boundary problems (the null value $\varphi = 0$ is at the boundary of the parameter space for H_1), indeterminate parameters (e.g., when the introgression probability $\varphi = 0$, the time of introgression becomes unidentifiable), and in particular multiple routes from the alternative hypothesis to the null hypothesis (e.g., fig 1c). While these do not invalid the definition and use of the Bayes factor for hypothesis testing, they pose challenges to the use of the S-D density ratio to calculate the Bayes factor. In this study, we show that each route from H_1 to H_0 can be used to calculate the Bayes factor via the S-D density ratio. When the condition on priors is satisfied over all routes, a combined estimate using all routes is also possible. Our simulation demonstrates that our theories can be implemented in practice, and the resulting Bayesian test has low false positives and high power for testing gene flow between sister lineages.

For parameter estimation, we see that the posterior surface under introgression (MSC-I) models may involve nearly flat ridges and multiple local peaks when there is no gene flow or when the rate of gene flow is low. There are also label-switching unidentifiability issues under the bidirectional model (BDI) (Yang and Flouri, 2022). When the rate of introgression is low, one should exercise care when interpreting simple summaries of the MCMC samples (such as posterior means and CIs) (fig. S3).

Here we note a few advantages of the Bayes factor, relative to Frequentist hypothesis testing such as the LRT (Yang, 2025). The Bayes factor penalizes parameter-rich models automatically. It works whether the compared models are nested or nonnested, and when the compared models have an irregular parameter space. The Bayes factor is also able to reject the alternative hypothesis with great force.

We note that many methods exist for calculating the marginal likelihood (see, for review, Fourment *et al.*, 2020). Numerically stable algorithms, such as path-sampling or thermodynamic integration (Ogata, 1989; Gelman and Meng, 1998; Lartillot and Philippe, 2006), stepping-stones (Xie *et al.*, 2011), and nested sampling (Skilling, 2006), require at least 50 runs of the MCMC algorithm for each of the two compared models (table 1 Fourment *et al.*, 2020). The D-S approach to computing the Bayes factor requires only one run of the MCMC algorithm under the alternative model of gene flow (H_1) (Dickey, 1971; Ji *et al.*, 2023), and involves $\sim$ 100 times less computation than those sample-based methods, offering a huge computational advantage. It is thus useful to extend the S-D approach to nonstandard conditions. We note that most applications of samples-based methods of marginal likelihood calculation in phylogenomics do not appear to compute the marginal likelihood to a high precision (precise to 0.1 log units, say, which translate to a factor of $e^{0.1} = 10\%$ in posterior model probabilities or posterior odds).

It is noteworthy that the irregularities discussed in this paper concern the test of gene flow between sister lineages under the discrete introgression model (MSC-I: UDI and BDI). Bayesian test of introgression (under either UDI or BDI) between non-sister lineages is not affected by those issues. Furthermore, the testing problem under MSC-M does not involve the complexities discussed in this paper. When $\varpi_{AB} = 0$ in H_1, the null value is at the boundary of the parameter space (which is not a problem for either the Bayes factor or the S-D approach for calculating it), but no other parameters become indeterminate. Neither does the problem of multiple routes for reducing H_1 to H_0 exist in the test under the MSC-M model. Even the bidirectional migration model in the case of two species (with both ϖ_{AB} and ϖ_{BA}) is identifiable with data of multiple samples per species per locus, unlike the corresponding MSC-I model, which shows two unidentifiable modes (Yang and Flouri, 2022).

Note also that the irregularities for the test of introgression between sister species affect the test or parameter estimation when introgression is absent or the rate is very low. At high rates, or when there is clear evidence of introgression and our objective is to

estimate parameters such as the introgression probability, the irregularities are not relevant. When the MCMC samples the rest of the parameter space in H_1, away from the three lines in figure 1d, different points in the space represent distinct biological scenarios and are identifiable. Nevertheless the information content in the data may be affected by species divergence time.

The theory developed in this paper applies as well if introgression is between sister lineages that are ancestral branches on the species phylogeny (fig. 9). Whether one or both of the sister lineages involved are ancestral, there are three routes for reducing the UDI introgression model (H_1) to the null model of no gene flow (H_0). For example, in comparison of the two models of figure 9a&b, the null point also consists of three lines or line segments, given by eq. 35 (fig. 1c). The same holds true for the test comparing the two models of figure 9c&d, in which case both sister lineages are ancestral. Similarly in the test under the BDI introgression model, there are five routes for reducing H_1 to H_0, as in the case where both involved species are extant species (eq. 38).

Alternative tests of gene flow between sister lineages

The irregularities of the testing problem discussed in this paper also impact Frequentist hypothesis testing such as the LRT as well, and indeed cause insurmountable difficulties for which no known solutions exist (Chernoff, 1954; Self and Liang, 1987). In the case where H_0 corresponds to H_1 with one parameter of interest at the boundary of the space, the null distribution for the LRT statistic is a 50:50 mixture of 0 and χ^2_1 (Chernoff, 1954). The problem of indeterminate parameters is harder, and in general the null distribution is unknown. The problem of multiple routes for reducing H_1 to H_0 does not appear to have been studied.

In population genetics, the programs ∂a∂i (Gutenkunst *et al.*, 2009) and FASTSIMCOAL2 (Excoffier *et al.*, 2013) calculate a composite likelihood for data of joint site frequency spectrum (SFS). The likelihood is composite as the method ignores the lack of independence among linked SNP sites, or the lack of independence among population pairs when multiple populations are analyzed. The use of composite likelihood ignoring correlation and the use of data summaries that are not sufficient statistics make it hard to define the null distribution of the test statistic; nonparametric resampling methods such as bootstrap are used instead.

Supplementary Material and Data Availability

Supplemental information is available at https://doi.org/10.5281/zenodo.xxxxxxxx. Supplementary data (sequence alignments and control files for running BPP are available through the Dryad Digital Repository at 10.5061/dryad.zkh1893pp.

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Conflict of Interest

None declared.

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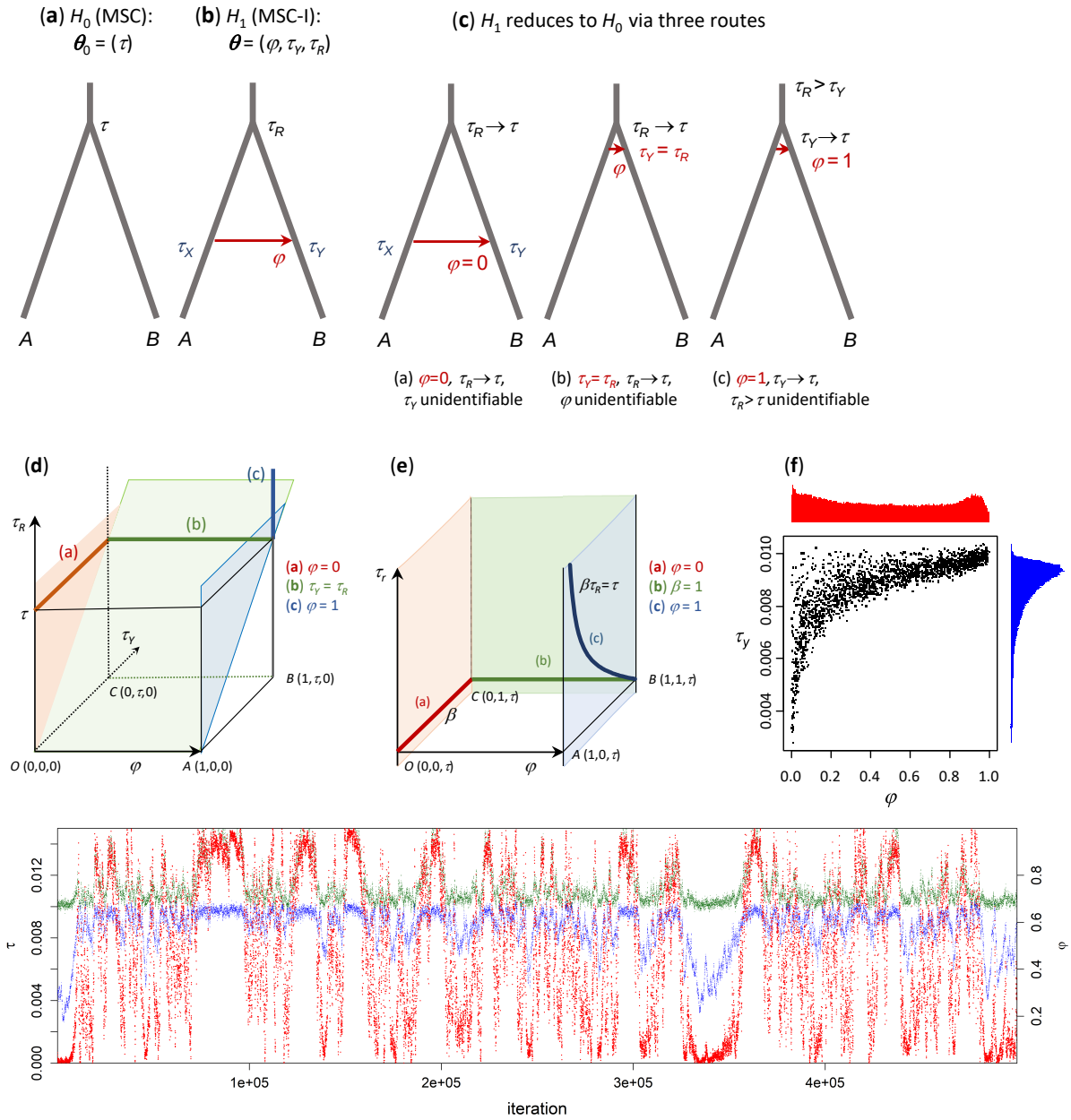

Fig. 1: (**a**, **b**) The null (H_0) and alternative (H_1) hypotheses in the test of (unidirectional) introgression from A to B. The parameter vector is $\boldsymbol{\theta}_0 = (\tau)$ under H_0 and $\boldsymbol{\theta} = (\varphi, \tau_Y, \tau_R)$ under H_1, with the population size parameter (θ) suppressed.
(**c**) H_1 reduces to H_0 via three routes (constraints): (a), (b), and (c) (table 1(i)).
(**d**) Parameter space of H_1, showing the null point as three lines (a), (b), (c), which correspond to the three routes of panel **c**, and the null space (the parameter space for H_0) as three planes.
(**e**) Reparametrization of H_1, with $\beta = \tau_Y/\tau_R$ used instead of τ_Y, so that $\boldsymbol{\theta}' = (\varphi, \beta, \tau_R)$.
(**f**) The 2-D scatter plot (for τ_Y and φ) and (**g**) trace plot (green for τ_R, blue for τ_Y and red for φ) from an MCMC sample generated in a BPP analysis of a dataset simulated under H_0 with $\tau = 0.01$ and $\theta = 0.005$. The dataset consists of 500 loci, with 20 sequences per locus (10 per species), and with 500 sites in each sequence. The data are analyzed under H_1, using the priors $\theta \sim G(2, 200)$, $\tau_R \sim G(2, 200)$, and $\varphi \sim U(0, 1)$. The run for 2×10^6 MCMC iterations took 8h34m using 35 cores on a server.

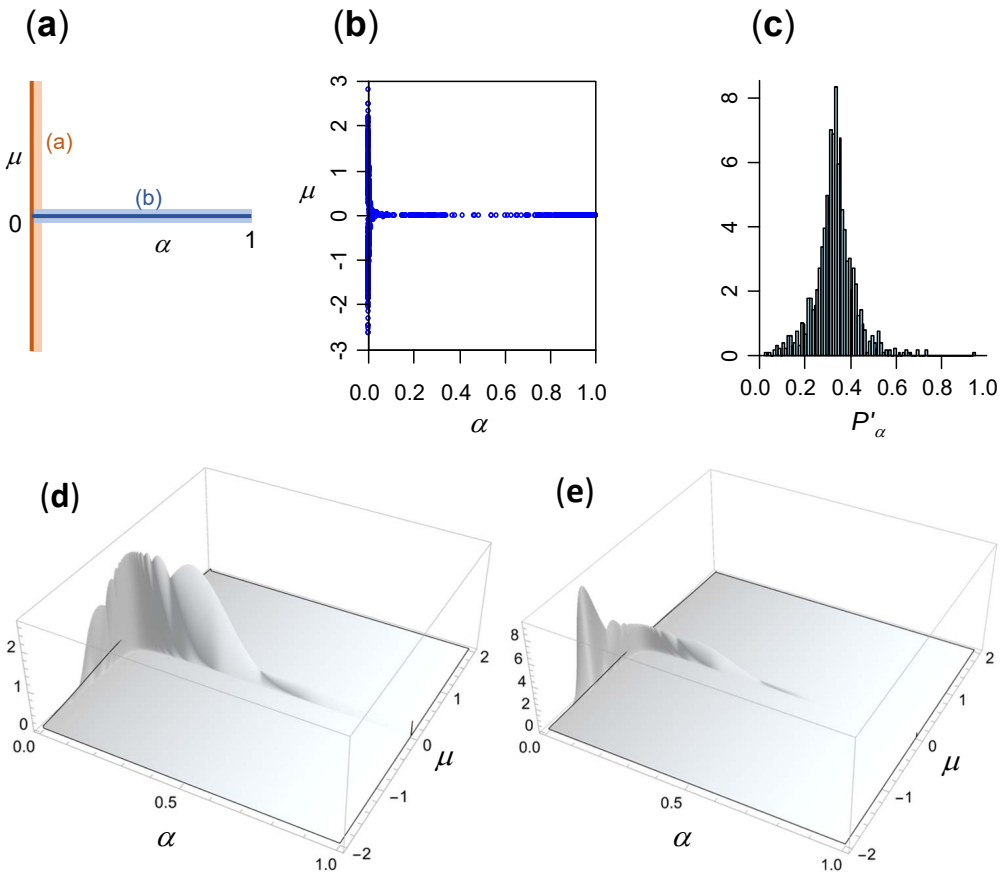

Fig. 2: (**a**) Parameter space for the Gaussian mixture model $H_1 : \alpha N(\mu, 1) + (1 - \alpha)N(0, 1)$ is the α–μ plane, with $0 < \alpha < 1$ and $-\infty < \mu < \infty$. The null point or null space for $H_0 : N(0, 1)$ is represented by the two axes: (a) $\alpha = 0$ (with μ unidentifiable) and (b) $\mu = 0$ (with α unidentifiable).
(**b**) MLEs $(\hat{\alpha}, \hat{\mu})$ under H_1 from 10^3 datasets (of size $n = 10^6$) simulated under H_0. The MLE corresponds to the highest ℓ in ten runs of the BFGS algorithm. Note that all points on the two axes have identical likelihood so that the MLE may be one unique point only if it is not on the axes.
(**c**) Histogram for posterior probability for segment (a), P_α, among datasets of panel **b**. Gaussian quadrature with 128 or 256 points in each dimension is used to calculate P_α, P_μ (eq. 24), with $\epsilon_\alpha = 0.02/\sqrt{12}, \epsilon_\mu = 0.05$, which are then rescaled to sum to one, $P'_\alpha = P_\alpha/(P_\alpha + P_\mu)$. Mathematica gives identical results for $n \leq 2000$ but encounters numerical problems for larger n. $P_\alpha + P_\mu > 0.8$ in 64% of datasets.
(**d**, **e**) Unnormalized posterior surface (eq. 18) for two datasets of size $n = 10^6$ simulated under H_0. In the first, the sample mean is $\bar{x} = -0.001931$, and the MLE under H_1 is $(\hat{\alpha}, \hat{\mu}) = (1, -0.001931)$ with the log likelihood (relative to that for H_0) $\Delta\ell = 1.864$. Multiple local peaks exist close to the α-axis, including (0, any) with $\Delta\ell = 0$, and (0.328984, −0.005867) and (0.791298, −0.002440) both with $\Delta\ell = 1.864$. In the second, the sample mean is $\bar{x} = -0.001830$. The MLE is (0.000927, −1.571305) close to the μ-axis, with $\Delta\ell = 4.381$, but local peaks exist close to the α-axis, such as (0.717088, −0.002553), (0.802798, −0.002280), and (0.909107, −0.002013) all with $\Delta\ell = 1.675$.

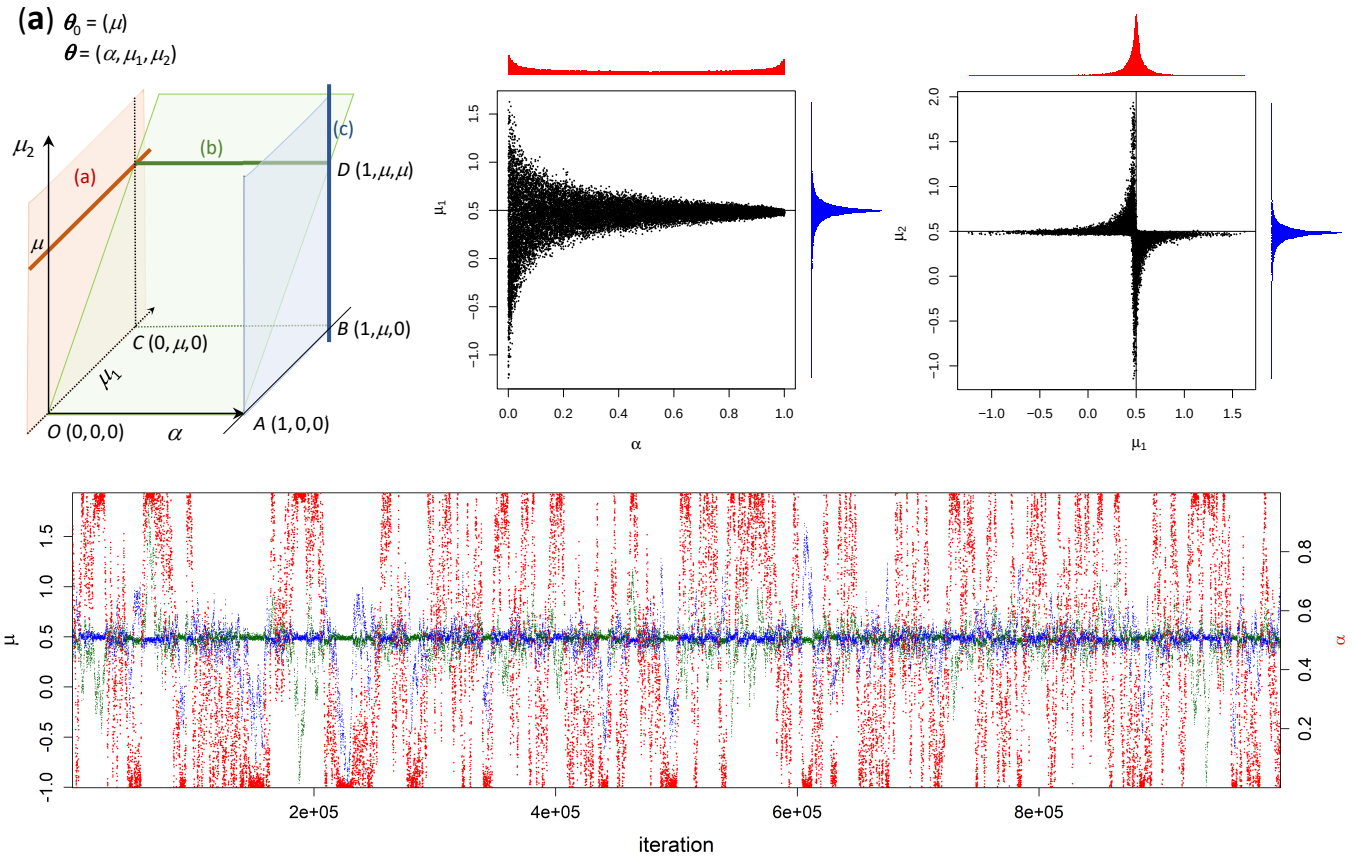

Fig. 3: (**a**) The 3-D parameter space for the Gaussian mixture model $H_1 : \alpha N(\mu_1, 1) + (1-\alpha)N(\mu_2, 1)$, with parameter vector $\boldsymbol{\theta} = (\alpha, \mu_1, \mu_2)$. The null model is $H_0 : N(\mu, 1)$. The null space corresponds to the three planes while the null point corresponds to three lines or line segments: (a) $\alpha = 0$ (with μ_1 unidentifiable and with $\mu_2 \to \mu$), (b) $\mu_1 = \mu_2$ (with $0 \le \alpha \le 1$ unidentifiable and with $\mu_1 = \mu_2 \to \mu$), and (c) $\alpha = 1$ (with μ_2 unidentifiable and with $\mu_1 \to \mu$). (**b**, **c**) 2-D scatter plots and (**d**) trace plots of α (red), μ_1 (blue) and μ_2 (green) from an MCMC analysis under H_1 of a dataset of size $n = 10^4$, simulated under H_0 with $\mu = 0.5$. The posterior means are $\alpha = 0.500$, $\mu_1 = 0.501$, $\mu_2 = 0.504$. There exists a lower mode at $\mu_1 = 0$ in the marginal of μ_1 (and similarly at $\mu_2 = 0$ in the marginal of μ_2), but these are not visible. Running time for 2×10^6 MCMC iterations is 32mins using one thread.

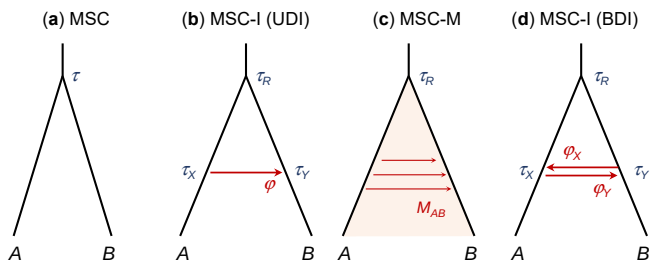

Fig. 4: (**a**) Unidirectional introgression model (MSC-I, UDI) with $A \to B$ (or $X \to Y$) introgression occurring at time $\tau_X = \tau_Y$ and introgression probability φ_Y. (**b**) Continuous migration model (MSC-M) with $A \to B$ migration at the population migration rate M_{AB} per generation. (**c**) Bidirectional introgression model (MSC-I, BDI) with introgression probabilities φ_X and φ_Y and introgression time $\tau_X = \tau_Y$. The MSC-I UDI and MSC-M models (**a**, **b**) are used to simulate data while all three models of gene flow (**b**-**d**) are used to analyze data.

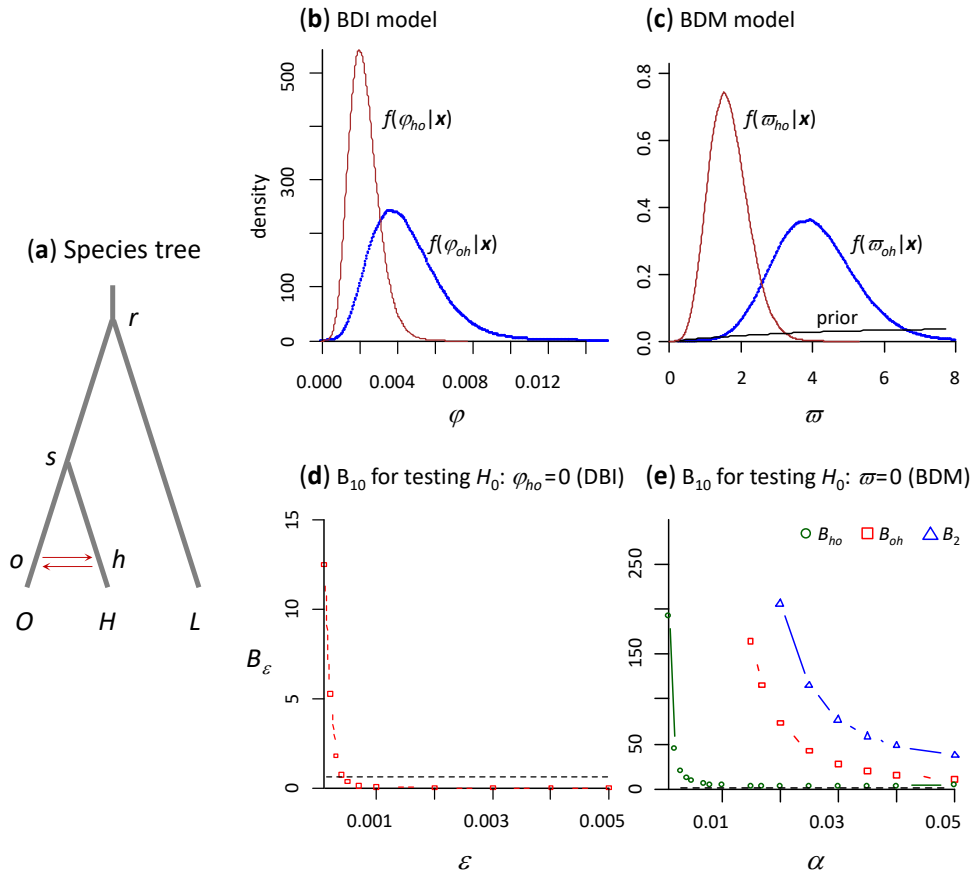

Fig. 5: (**a**) Phylogeny for three Baja California lizard species, *Sceloporus orcutti* (O), *S. hunsakeri* (H), and *S. licki* (L), with gene flow between O and H. (**b**) Posterior densities for introgression probabilities φ_{oh} and φ_{ho} obtained in BPP analysis of the ddRAD data under the BDI model. The prior, $\varphi \sim U(0,1)$, is invisible. (**c**) Posterior densities for migration rates $(\varpi_{oh}, \varpi_{ho})$ under the BDM model. The prior $\varpi \sim G(2, 0.1)$ is shown as well. See table 3 for estimates of other parameters under the two models. (**d**) Approximate Bayes factor $(B_{10,\epsilon})$ for testing $H_0 : \varphi_{ho} = 0$ against $H_1 : \varphi_{ho} \neq 0$, with φ_{oh} treated as a nuisance parameter, calculated using an MCMC sample under BDI of panel **b**. (**e**) $B_{10,\epsilon}$ for testing $H_0 : \varpi_{ho} = 0$; $H_0 : \varpi_{oh} = 0$; or $H_0 : \varpi_{ho} = 0, \varpi_{oh} = 0$ using the MCMC sample under BDM of panel **d**, plotted against the tail probability of the prior, $\alpha = \mathbb{P}(\varpi < \epsilon)$.

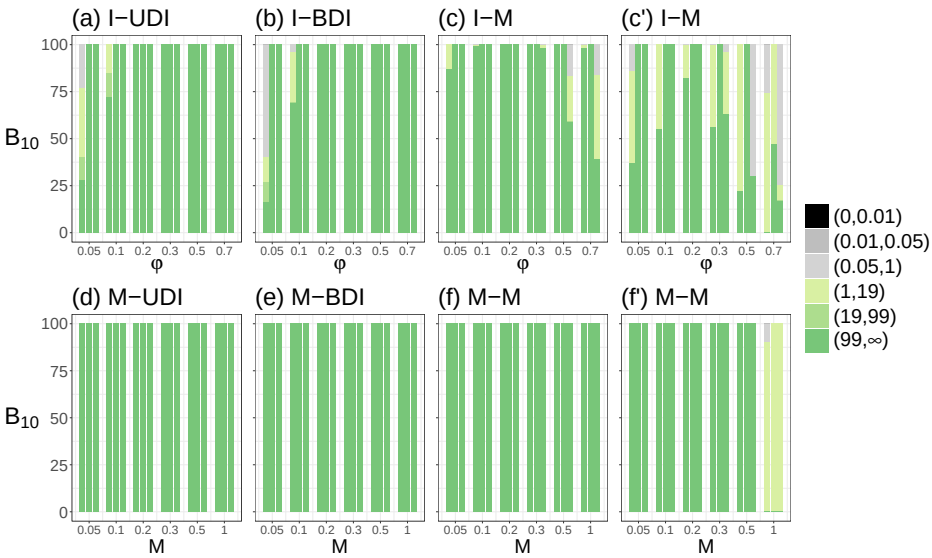

Fig. 6: Power of the Bayesian test of gene flow between sister lineages. Bayes factor (B_{10}) calculated using data simulated under the MSC-I or MSC-M models of figure 4b&c, and analyzed under the three models of figure 4b–d. The setting is in the format of simulation model-analysis model: for example, in M-UDI, data are simulated under MSC-M and analyzed under MSC-I (UDI) (fig. 4b&c). The MSC-M model was implemented under two priors, $\varpi \sim G(2, 0.01)$ with mean 200 in panels **c** & **f**, and $\varpi \sim G(2, 1)$ with mean 2 in panels **c′** & **f′**. The stacked bar plots show the proportions of datasets (out of 100 replicates) in which B_{10} falls in different intervals: 0–0.01 (strong rejection of gene flow), 0.01–0.05 (rejection of gene flow), 0.05–1 (preference for no gene flow), 1–19 (preference for gene flow), 19–99 (support for gene flow), and 99–∞ (strong support for gene flow). The three bars in each group correspond to three divergence levels, with $\tau_R = \frac{1}{2}\theta$ (very shallow), θ (shallow), and 10θ (deep). B_{10} is calculated via the S-D density ratio with $\epsilon_\varphi, \epsilon_\beta$ in MSC-I and ϵ_ϖ in MSC-M set to 0.01σ, where σ is the prior standard deviation; use of 0.005σ and 0.05σ produces very similar results.

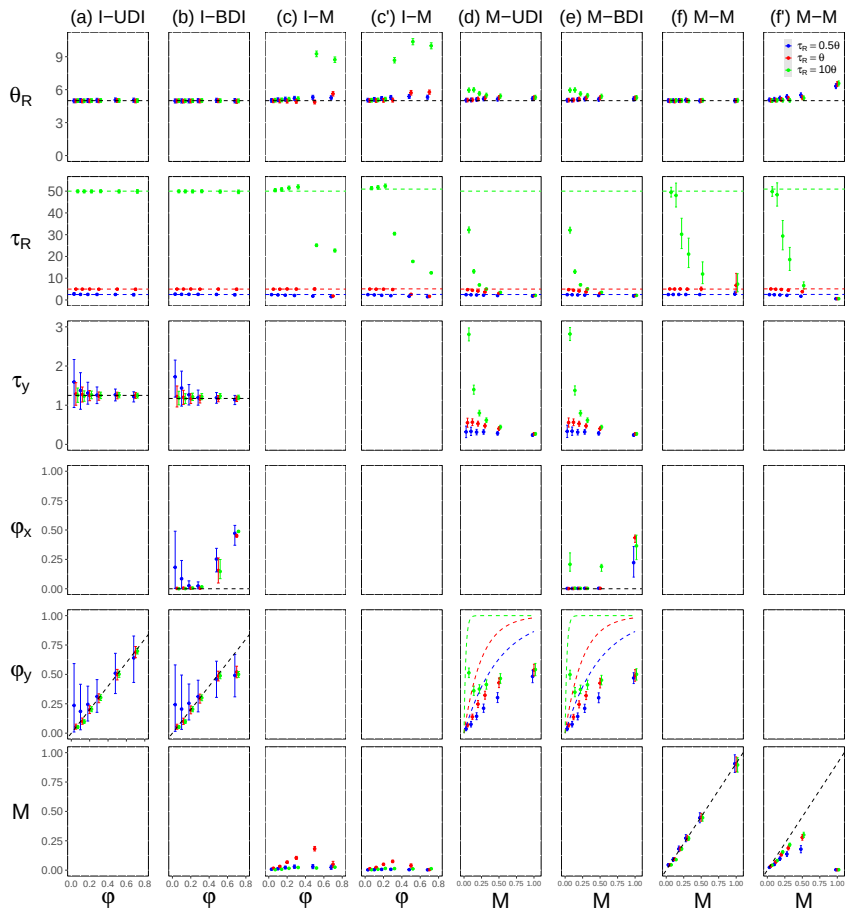

Fig. 7: Average posterior means and 95% HPD CIs of parameters in BPP analysis of data of figure 6. See legend to figure 6. Dotted lines in the plots for φ_y in the M-UDI and M-BDI settings represent the expected amount of gene flow in the true MSC-M model (φ_0 in eq. 41).

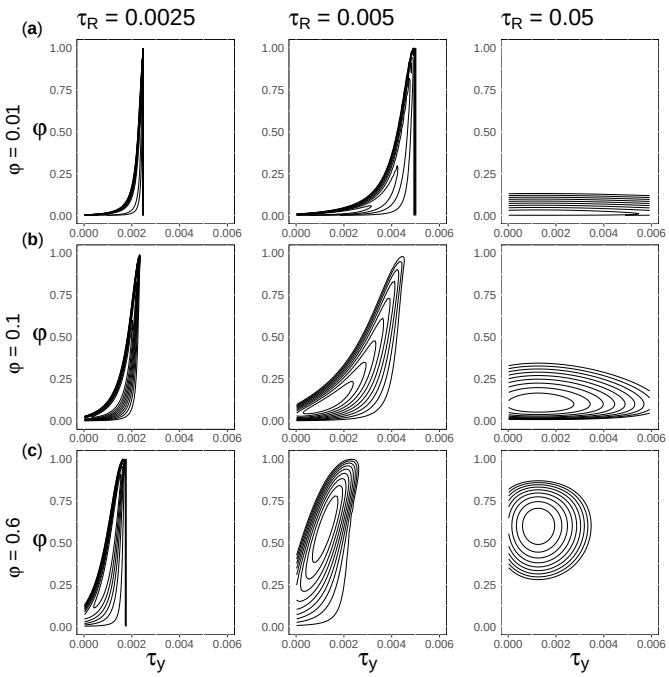

Fig. 8: Contour plot of the log likelihood (eq. 42) as a function of τ_Y and φ under the MSC-I UDI model (fig. 4b) for the limiting case of $L = \infty$ loci, generated using the true parameter values $\varphi = 0.01, 0.1$ or 0.6, and $\tau_R = \frac{1}{2}\theta$, θ, or 10θ, with $\theta = 0.005$ and $\tau_Y = \frac{1}{4}\theta$. Each locus consists of two sequences (one from each species), summarized as the number of differences at n sites. Here $\theta = 0.005$ is fixed at the true value, while τ_R is optimized given φ and τ_Y; fixing τ_R at the true value produces nearly identical results.

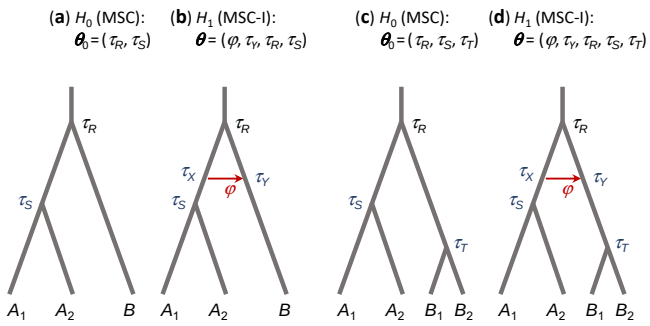

Fig. 9: (**a**, **c**) Null (H_0) and (**b**, **d**) alternative (H_1) hypotheses for testing (unidirectional) introgression that involves one or two ancestral (sister) species. Population-size parameters (θ) are not shown.

Table 1: Parameters of interest (ω), nuisance parameters (λ), and indeterminate parameters (ξ) and condition on priors in Bayesian test of introgression

Segment	Parameters of interest $\omega = \omega_0$	Indeterminate ξ	Nuisance λ	Condition on λ (eq. 4)
(**i**) Unidirectional introgression (UDI, fig. 1c)				
(a)	$\varphi = 0$	(τ_Y)	(τ_R, θ)	yes
(b)	$\beta = \frac{\tau_Y}{\tau_R} = 1$	(φ)	(τ_R, θ)	yes
(c)	$\varphi = 1$	(τ_R)	(τ_Y, θ)	no
(**ii**) Bidirectional introgression (BDI)				
(a)	$\varphi_X = 0, \varphi_Y = 0$	(τ_Y)	(τ_R, θ)	yes
(b)	$\varphi_X = 1, \varphi_Y = 1$	(τ_Y)	(τ_R, θ)	yes
(c)	$\beta = \frac{\tau_Y}{\tau_R} = 1$	(φ_X, φ_Y)	(τ_R, θ)	yes
(d)	$\varphi_X = 0, \varphi_Y = 1$	(τ_R)	(τ_Y, θ)	no
(e)	$\varphi_X = 1, \varphi_Y = 0$	(τ_R)	(τ_Y, θ)	no

Note.— For UDI, in segment (c) nuisance parameter τ_Y in H_1 is mapped onto τ in H_0 (fig. 1a&b). For BDI, segments (a), (b), (d), and (e) are lines, while segment (c) is a plane, and in segments (d) and (e), nuisance parameter τ_Y in H_1 is mapped to τ_R in H_0. In last column, 'yes' means that the condition on priors (eq. 4) holds for the segment and 'no' means it does not.

Table 2: Bayes factor (B_{10}) for testing introgression in 100 replicate datasets simulated under the null model of no gene flow (H_0, MSC, fig. 4a), and analyzed under models of gene flow (H_1, MSC-I and MSC-M, fig. 4b–d)

	(**a**) MSC-I, UDI			(**b**) MSC-I, BDI			(**c**) MSC-M		
B_{10}	$\frac{1}{2}\theta$	θ	10θ	$\frac{1}{2}\theta$	θ	10θ	$\frac{1}{2}\theta$	θ	10θ
(**i**) With $\epsilon_\varphi, \epsilon_\varpi$ set at 0.005σ									
(0, 0.0101)	0	0	49	0	0	2	0	0	96
(0.0101, 0.0526)	1	4	31	3	0	34	0	4	4
(0.0526, 1)	89	89	20	90	94	62	69	92	0
(1, 19)	10	7	0	7	5	2	20	4	0
(19, 99)	0	0	0	0	1	0	0	0	0
(99, ∞)	0	0	0	0	2	0	7	0	0
(**ii**) With $\epsilon_\varphi, \epsilon_\varpi$ set at 0.01σ									
(0, 0.0101)	0	0	45	0	0	0	0	0	95
(0.0101, 0.0526)	0	3	27	0	0	34	0	2	5
(0.0526, 1)	90	92	28	93	95	66	80	95	0
(1, 19)	10	5	0	7	4	0	18	3	0
(19, 99)	0	0	0	0	1	0	0	0	0
(99, ∞)	0	0	0	0	0	0	2	0	0
(**iii**) With $\epsilon_\varphi, \epsilon_\varpi$ set at 0.05σ									
(0, 0.0101)	0	0	31	0	0	1	0	0	94
(0.0101, 0.0526)	1	0	34	3	1	45	0	0	6
(0.0526, 1)	92	95	35	90	95	53	79	97	0
(1, 19)	7	5	0	7	4	1	20	3	0
(19, 99)	0	0	0	0	0	0	0	0	0
(99, ∞)	0	0	0	0	0	0	1	0	0

Note.– B_{10} is calculated using the S-D density ratio with $\epsilon_\varphi = \epsilon_\beta$ (in MSC-I) and ϵ_ϖ (in MSC-M) set at $0.005\times, 0.01\times, 0.05\times\sigma$, where σ is the prior standard deviation; i.e., $\sigma = \frac{1}{\sqrt{12}} = 0.28867$ for $\varphi, \beta \sim$ U(0, 1) and $\sigma = 1.414$ for $\varpi \sim$ G(2,1). The cutoffs, $B_{10} = 0.0101, 0.0526, 1, 19$, and 99, correspond to the posterior probability for the model of gene flow $\mathbb{P}(H_1|\boldsymbol{x}) = 0.01, 0.05, 0.5, 0.95$, and 0.99, respectively.

Table 3: Posterior means and 95% HPD CIs for parameters under the BDI and BDM models obtained from BPP analyses of the lizard data

Parameters	MSC-I (BDI)	MSC-M (BDM)
τ_r	0.0079 (0.0075, 0.0084)	0.0080 (0.0076, 0.0084)
τ_s	0.0074 (0.0070, 0.0079)	0.0076 (0.0071, 0.0081)
$\tau_o = \tau_h$	0.0003 (0.0001, 0.0005)	
θ_O	0.0079 (0.0075, 0.0083)	0.0079 (0.0075, 0.0083)
θ_H	0.0034 (0.0032, 0.0037)	0.0034 (0.0031, 0.0036)
θ_L	0.0055 (0.0052, 0.0059)	0.0055 (0.0052, 0.0059)
θ_r	0.0073 (0.0064, 0.0082)	0.0071 (0.0062, 0.0080)
θ_s	0.0046 (0.0007, 0.0088)	0.0046 (0.0004, 0.0096)
φ_{oh}	0.0044 (0.0013, 0.0079)	
φ_{ho}	0.0022 (0.0008, 0.0038)	
ϖ_{oh}		4.0632 (1.9092, 6.2317)
ϖ_{ho}		1.6610 (0.6325, 2.7243)
M_{oh}		0.0034 (0.0017, 0.0053)
M_{oh}		0.0033 (0.0013, 0.0054)

Note.— Under the MSC-M model, the prior $\varpi \sim G(2, 0.01)$ is used; use of $G(2, 1)$ produced identical results at this level of precision.