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EPISODIC CHROMOSOMAL EVOLUTION IN *PLANIPAPILLUS* (ONYCHOPHORA: PERIPATOPSIDAE): A PHYLOGENETIC APPROACH TO EVOLUTIONARY DYNAMICS AND SPECIATION

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Abstract.—*Planipapillus*, a clade of onychophorans from southeastern Australia, exhibits substantial chromosomal variation. In the context of a robust phylogeny based on nuclear and mitochondrial sequence data, we evaluate models of chromosomal evolution and speciation that differ in the roles assigned to selection, mutation, and drift. Permutation tests suggest that all chromosome rearrangements in the clade have been centric fusions and, on the basis of parsimony and maximum-likelihood methods with independent estimates of branch lengths, we conclude that at least 31 centric fusions have been fixed in *Planipapillus*. A likelihood-ratio test approach, which is independent of our point estimates of ancestral states, rejects an evolutionary model in which the mutation rate is constant and centric fusions are effectively neutral. In contrast to the nucleotide sequence data, which are consistent with neutrality and rate constancy, centric fusions in *Planipapillus* are underdominant, spontaneous fusion rates vary among lineages, or both. We predict an inverse relationship between rates of chromosomal evolution and historical population size. Chromosomal evolution may play a role in speciation in *Planipapillus*, both by interactions between centric fusions with monobrachial homology and by the accumulation of multiple weakly underdominant fusions.

Key words.—Centric fusion, character-state reconstruction, chromosomal evolution, chromosomal speciation, neutrality, Onychophora, underdominance.

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The roles of natural selection, drift, and mutation in shaping the evolution of karyotypes and the relationship between chromosomal evolution and speciation are topics that have been largely refractory to broad generalizations. We introduce a phylogenetic approach to unravel the evolutionary dynamics of karyotypes and take advantage of the unique attributes of *Planipapillus* Reid 1996, the lawn-headed onychophorans (velvet worms, or peripatus) of southeastern Australia, as a model system for the study of chromosomal evolution. In common with the majority of Australian onychophorans, *Planipapillus* are predators on small invertebrates and inhabit the moist interiors of rotting logs. In every population, the fundamental number of the karyotype (the number of arms) is 40; the whole chromosome number, however, varies among localities, from $2n = 40$, an all-telocentric karyotype, to $2n = 20$, an all-metacentric karyotype (Rowell et al., 2002). Changes in diploid number in *Planipapillus* can thus be ascribed to Robertsonian, or whole-arm, rearrangements. The phylogenetic approach allows provisional answers to a series of questions about *Planipapillus* chromosomal evolution: What is the nature of the chromosome rearrangements? How many have there been? What are the ancestral karyotypes? What are the roles of selection, drift, and mutation in the evolution of these karyotypes? How do these rearrangements relate to speciation?

Evolutionary Dynamics

The evolution of chromosome rearrangements has received a great deal of theoretical treatment. In general, structural heterozygotes often produce aneuploid gametes as a result of nondisjunction at anaphase or duplication and/or deficiency products due to unequal recombination. As a consequence, chromosome rearrangements have been modeled as a classic example of underdominance (heterozygote disadvantage), and their evolution has been treated as a peak shift on a fitness landscape (Wright 1941; Lande 1979, 1985; Hedrick 1981; Hedrick and Levin 1984; Coyne 1989; Barton and Rouhani 1991; Spirito 1992; Michalakis and Olivieri 1993). This view is corroborated by the rarity of chromosomal polymorphisms (White 1973). Recently, however, the uniform underdominance of chromosome rearrangements has been questioned (Nachman and Myers 1989; Bidau 1990; Rowell 1990, 1991; Coyne et al. 1991, 1997; Arévalo et al. 1994; Reed et al. 1995; Orr 1996). These alternative views hold that fixed rearrangements are either selectively favored or that they are neutral and fixed solely by drift.

Robertsonian rearrangements, because they do not necessarily interfere with recombination or segregation, are often characterized as potentially neutral rearrangements. The ability of heterozygotes for a single fusion to undergo balanced segregation, or at least show no fitness deficit, holds true in a number of groups, including populations of iguanid lizards (Porter and Sites 1985), cattle (Rangel-Figueiredo and Iannuzzi 1993), sheep (Bruére and Ellis 1979), and grasshoppers (Bidau and Mirol 1988). In other cases, however, Robertsonian rearrangements are associated with decreased heterozygote fitness, consistent with underdominance (e.g., mice: Hauffe and Searle 1998; Britton-Davidian et al. 2000; Castiglia and Capanna 2000; shrews: Searle and Wójcik 1998; and cattle: Weber et al. 1989). Robertsonian rearrangements may also be advantageous, either via some benefit associated with nuclear organization (Imai et al. 1994) or by the reorganization of linkage groups due to novel chiasma distributions associated with structural heterozygosity (Rowell 1991).

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Most efforts to unravel the selective history of chromosome rearrangements have relied on inferences from the contemporary selective regime, especially in the context of hybrid zones or laboratory crosses. Unfortunately, several factors are confounded in these studies (Sites and Reed 1994; Sites 1995; Coyne and Orr 1998). Most importantly, effects of chromosomal differences are difficult to distinguish from the effects of genic differences, both of which may have accumulated subsequent to population divergence. Different selective regimes in different environments may also cause chromosomal differences that affect hybrid fitnesses. In sum, data on present-day hybrid fitness is generally insufficient to infer the effects of chromosomal differences in ancestral populations.

Extensive laboratory or field studies of *Planipapillus* are not currently feasible. However, in conjunction with a well-resolved species-level phylogeny with estimates of branch lengths, we are able to test a model of neutral chromosomal evolution in a manner that would not be possible with crosses or hybrid zone studies.

Such tests are possible because of a signal quality of the *Planipapillus* karyotype dataset: It includes a large number of rearrangements of the same sort. By pooling these observations and treating ensemble properties such as mean rate, we can apply the theory and methods developed for molecular evolution and molecular population genetics as null models against which to test our empirical data. The key assumption is that the rearrangements constitute a homogenous class, such that with respect to mutation and selection each rearrangement is equivalent. Analogous assumptions are made in most molecular evolution studies, for example, that observations can be pooled in categories such as synonymous substitutions, second codon positions, rRNA stems, transmembrane domains, and so forth. The assumption that all rearrangements are equivalent obviously does not hold in general, but it appears reasonable when restricted to Robertsonian rearrangements in *Planipapillus*. As we argue below, all rearrangements in *Planipapillus* appear to be centric fusions. The pattern of karyotype diversity shows that each telocentric chromosome is capable of involvement in fusions, which have occurred independently in many lines. Studies of other taxa with similar patterns of karyotype diversity (e.g., Australian *Rattus*, Baverstock et al. 1986; *Rhogeesa* bats, Baker et al. 1985; huntsman spiders, Rowell 1990; and especially *Mus musculus*, Capanna 1982; Redi et al. 1990), have shown that fusion mutation and fixation may be random with respect to chromosome identity.

The role of selection is then examined with reference to a null model of neutral evolution. We use a likelihood-ratio test approach to evaluate the hypothesis that rearrangements have been neutral and that the rate of rearrangement mutations has been constant. If mutation is a stochastically constant Poisson process over the whole phylogeny, and individual fusions are neutral, the rate of fixation of rearrangements will be equal to the rearrangement mutation rate (Kimura 1983). Fixation of neutral rearrangements should then follow a Poisson process (Ohta and Kimura 1971; Langley and Fitch 1974; Kimura 1983; Takahata 1987; Gillespie 1989, 1991; Goldman 1994; Ohta 1995; Zeng et al. 1998; Cutler 2000), and rearrangements should be Poisson distributed on the *Planipapillus* phylogeny. Some caveats of this approach are detailed in the Discussion.

Speciation

Classical models of chromosomal speciation relied on the fixation of underdominant rearrangements to cause reproductive isolation; the difficulty with these theories is that the fixation of underdominant karyotypes is extraordinarily unlikely because of selection against heterozygotes. As noted above, many recent authors have rejected this model of chromosomal evolution, and hence speciation, as being so improbable as to be impossible (e.g., Orr 1996; Coyne et al. 1997).

Two models have established circumstances under which neutral or advantageous rearrangements may contribute to speciation. In one, Rieseberg (2001) argued that rearrangements linked to isolation genes may, by suppressing recombination, protect large regions of the genome from gene flow and therefore facilitate speciation. The other model is specific to the case of centric fusions and requires no involvement from isolation genes (Capanna 1982; Baker and Bickham 1986). Central to this model is the assumption that centric fusions do not impede balanced segregation; at meiosis, heterozygotes form trivalents consisting of two telocentric chromosomes paired with the homologous arms in a metacentric. These generally segregate correctly, resulting in little or no heterozygote disadvantage. In the absence of fitness consequences, random drift alone will determine whether new fusions become fixed, and populations fixed for a given fusion will not be reproductively isolated from populations in which the rearrangement is absent. When we consider multiple fusions, however, the potential arises for the formation of reproductive barriers. If in two populations drift results in the fixation of different fusions with one chromosome in common (e.g., a fusion of chromosomes 1 and 2 in one population one and 1 and 3 in another), hybrids will form quadrivalents at meiosis and segregation efficiency may be impaired. When more chromosomes show such monobrachial homology, even more complicated meiotic configurations result and balanced segregation becomes increasingly less likely. Infertility of heterozygotes for monobrachially homologous rearrangements has been demonstrated repeatedly (e.g., Baverstock et al. 1983; Castiglia and Capanna 2000). Speciation by centric fusions with monobrachial homology is analogous (although not identical, see Discussion) to the Dobzhansky-Muller model of genic speciation (Orr 1995, 1996; Coyne et al. 1997, 2000); individual mutations may be fixed without underdominance, but interactions among mutations at different loci (or, in this case, chromosomes) may result in reduced hybrid fitness. A reconstruction of the history of *Planipapillus* fusions is a first step toward evaluating the role of chromosome rearrangements in the clade's evolution.

Materials and Methods

Phylogeny and Karyotypes

Planipapillus localities, karyotypes, and sample sizes are described in Rowell et al. (2002). Because most *Planipapillus* species are not formally named, we use numbers and locality

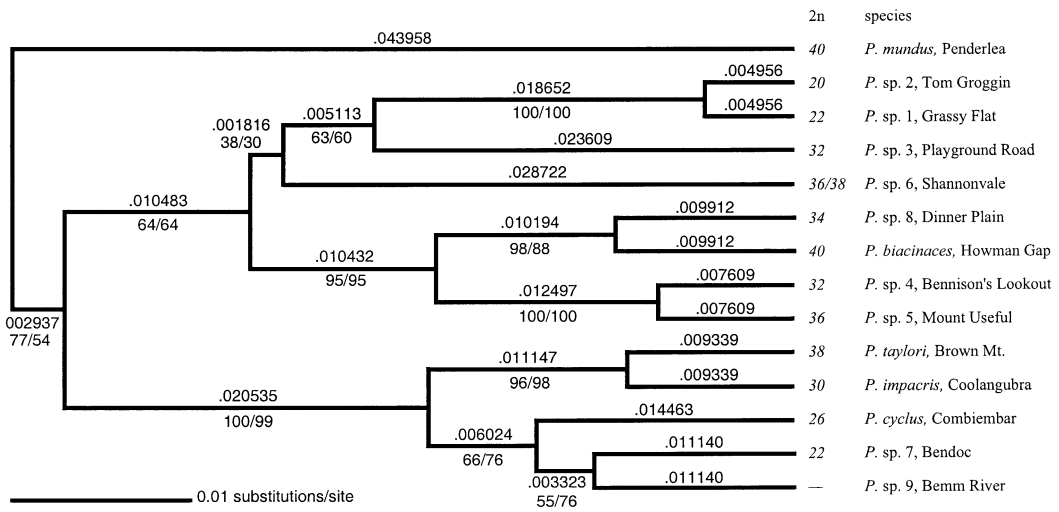

FIG. 1. Phylogram showing maximum-likelihood estimates of branch lengths, measured in substitutions per site. Also shown is support for each branch measured by nonparametric bootstrap analysis (parsimony and likelihood) of the concatenated dataset (Rockman et al. 2001), and, at the tips of the branches, the observed diploid numbers for each species.

names to represent some species, following Rockman et al. (2001). Outgroup taxa from Rockman et al. (2001) have karyotypes that differ substantially in diploid number, fundamental number, size distribution, and presence of differentiated sex chromosomes (D. M. Rowell, unpubl. data), and we are thus unable to use these outgroup karyotypes in our analysis. Given our symmetry assumptions and use of an ultrametric (clocklike) tree, detailed below, the lack of an outgroup has no impact on our ability to infer the karyotype at the root of the tree. We recovered two karyotypes from *P.* sp. 6, Shannonvale, and this taxon was treated as polymorphic in all analyses.

We use the *Planipapillus* phylogeny of Rockman et al. (2001), derived from partial sequences of two mitochondrial genes, 12S rRNA and COI, and of a nuclear intron from the *fushi tarazu* gene. The phylogeny is well supported by the concatenated data under both parsimony and likelihood criteria; bootstrap support for the tree, derived from Rockman et al. (2001), is shown here in Figure 1. We treat the topology as known. Branch lengths were estimated from the concatenated dataset of the three gene sequences (1152 sites) by maximum likelihood, using PAUP* (Swofford 1998). The likelihood model employed is Kimura's (1981) three-substitution-type model, modified to incorporate unequal equilibrium nucleotide frequencies. In addition, among-site rate variation was accommodated with gamma-distributed rates and a proportion of invariable sites. This K3STf + Γ + I model was identified by hierarchical likelihood-ratio tests (Posada and Crandall 1998) as the best-fit model for analysis of the concatenated sequences (Rockman et al. 2001). All parameters were simultaneously estimated by maximum likelihood on the favored topology (π_A = 0.35127, π_C = 0.09581, π_G = 0.12560, α = 0.67010, pInv = 0.57742; rate ratios: A-C type transversions = 1, A-T type transversions = 2.20269, transitions = 6.54649). The branch lengths were tested for conformity to a molecular clock by means of likelihood-ratio tests with 12 degrees of freedom (Felsenstein 1981). For the 14 *Planipapillus* taxa, the clock assumption cannot be rejected (P = 0.43), and we therefore estimated branch lengths under the constraint of a molecular clock. Because we lack karyotype data for *P.* sp. 9, Bemm River, this branch was omitted from subsequent analysis. The topology and branch lengths are shown in Figure 1.

Reconstructing Karyotype Evolution

Although biologists have been using phylogenies to reconstruct ancestral states for decades, only recently has the extraordinary difficulty of this enterprise been appreciated and have statistical methods been developed (Maddison and Maddison 1992; Frumhoff and Reeve 1994; Strathmann and Eernisse 1994; Schluter et al. 1997; Cunningham et al. 1998; and see the symposium papers in *Systematic Biology* 48(3), 1999). Historically, reconstructions of karyotype evolution have either used unweighted parsimony or have applied a priori transition series. We have decomposed the reconstruction into two steps: (1) estimating the transition symmetry, that is, the relative probabilities of the two relevant types of Robertsonian change, centric fusions versus centric fissions; and (2) assigning reconstructed karyotypes to ancestral nodes.

We employ several phylogenetic approaches to test our a priori assumption of asymmetry, specifically that all Robertsonian rearrangements in *Planipapillus* are centric fusions and none is due to fission. Our karyotype data take the form of an ordered character with 11 states ranging from $2n$ = 40 to $2n$ = 20. For symmetry analyses involving data randomization, 1000 equiprobable random trees and 1000 permutations of the observed states among the terminal taxa were generated in MacClade (Maddison and Maddison 1992).

We use both parsimony and likelihood methods to assign karyotypes to ancestral nodes, in each case constraining the analyses in accord with our symmetry assumptions. MacClade was used to find the parsimony reconstruction. Maximum-likelihood reconstructions (Pagel 1999) were identified by exhaustively calculating the likelihood for each of the

4692 possible reconstructions. We consider two models of evolution (Langley and Fitch 1974). In the first case, fusions occur according to a Poisson process with a single mean rate λ over the whole tree. The likelihood of observing x_i rearrangements on a branch with length t_i is

$$L_i = \frac{e^{-\lambda t_i}(\lambda t_i)^{x_i}}{x_i!}. \quad (1)$$

The likelihood of reconstruction j is the product of the likelihoods of each reconstruction branch:

$$L_j = \prod_{i=1}^{branches} L_i. \quad (2)$$

Taking the natural logarithm of equation (2), differentiating, and setting the result equal to zero, we can show that the maximum-likelihood estimator of λ for each reconstruction is given by

$$\hat{\lambda} = \sum_{i=1}^{branches} x_i \Big/ \sum_{i=1}^{branches} t_i, \quad (3)$$

the total number of fusions divided by the length of the tree. A second approach to maximum-likelihood reconstruction abandons the single rate assumption and allows each branch its own rate, λ_i. From equation (3), the maximum-likelihood estimator of each λ_i is $\hat{\lambda}_i = x_i/t_i$, and the branch likelihood in equation (1) reduces to

$$L_i = \frac{e^{-x_i} x_i^{x_i}}{x_i!}. \quad (4)$$

The reconstruction likelihood is again found by equation (2). For our data this unconstrained model is a 24-rate model. We treat each branch as an independent observation, and consequently tree topology does not enter into the likelihood calculations, except insofar as our branch length estimates are conditioned on the maximum-likelihood tree. Because the numbers of rearrangements on each branch, x_i, are fixed for each reconstruction, the branch length estimates, t_i, are the sole source of error in the estimates of $\hat{\lambda}$ and L_i.

Testing Models of Karyotype Evolution

We use a likelihood-ratio test of the hypothesis that karyotype evolution in *Planipapillus* follows a constant rate Poisson process (Langley and Fitch 1974; Pagel 1997; Mooers et al. 1999). The test statistic δ is based on the sum of likelihoods over all possible reconstructions and is therefore independent of our assignments of ancestral karyotypes:

$$\delta = -2 \ln\left(\sum_{j=1}^{reconstructions} L_j^{1-rate} \Big/ \sum_{j=1}^{reconstructions} L_j^{24-rate} \right). \quad (5)$$

A confidence interval for δ, accommodating the sampling error component of branch length estimation error, was estimated by a nonparametric bootstrap approach similar to that of Baldwin and Sanderson (1998). First, the Seqboot module of PHYLIP version 3.573 (Felsenstein 2000) was used to generate 100 bootstrap data matrices from the Rockman et al. (2001) sequences. From each bootstrap matrix, branch lengths were estimated by PAUP* for the Rockman et al. (2001) tree, holding the model parameters constant at the values indicated above. For each set of bootstrap branch lengths, the likelihoods under the one-rate and 24-rate models were calculated for each of the possible karyotype reconstructions, and δ was calculated as above.

Because the two likelihood models, one-rate and 24-rate, are nested, δ should be distributed as a χ^2 random variable with degrees of freedom equal to the difference in the number of free parameters. In phylogenetic contexts, however, likelihood-ratio test statistics are not always well behaved (Goldman 1993). We have used Monte Carlo simulations to generate a null distribution for the test statistic (Goldman 1993). We used Seq-Gen version 1.1 (Rambaut and Grassly 1997) to generate 1000 datasets at each of six rates, λ, such that the expected number of rearrangements over the tree, given by $\lambda \sum_{i=1}^{branches} t_i$, was 31, 35, 39, 43, 47, and 51. δ was then calculated for each simulation.

RESULTS

Direction of Karyotypic Change

Diploid number variation in *Planipapillus* can be explained in terms of Robertsonian rearrangements, specifically centric fusions and centric fissions. Parsimony character mapping treats fusions and fissions as equally likely, but we have a priori reasons to believe that all rearrangements in *Planipapillus* are fusions (Rowell et al. 2002). We use phylogenetic methods to seek corroboration for the fusions-only assumption.

One approach to the study of directional biases in character evolution is the evaluation of asymmetric step matrices (Omland 1997; Lee and Shine 1998; Ree and Donoghue 1998). Parsimony reconstructs character change by minimizing the cost of character changes over the tree. Costs are assigned by means of a step matrix (Maddison and Maddison 1992), which designates the cost of a change from any one state to any other. The usual approach is to assume a symmetrical step matrix, such that gains and losses are equally costly. Parsimony reconstruction using a symmetric step matrix gives an estimate of relative numbers of gains and losses on the tree, and subsequent consideration of asymmetric step matrices permits an assessment of the differences in cost that would be necessary to alter the proportion of changes in each direction. These cost differences are not readily interpretable in statistical terms.

Under the assumption of equal costs for fusions and fissions, there are 24 most parsimonious reconstructions, each requiring 27 rearrangements. The ratio of fusions to fissions on these reconstructions ranges from 5.75 to 1.08, with a mean of 2.18 (18.5 fusions and 8.5 fissions). When the same calculation is performed for 1000 random trees, the average mean ratio is 0.97 (SD = 0.34), with fissions slightly in the majority; a mean value as fusion-biased as 2.18 has a probability of 0.005. The preponderance of fusions in every reconstruction is thus a property of the reconstruction on the estimated tree and not a result of character-state bias (Collins et al. 1994; Ree and Donoghue 1998).

The excess of fusions under a symmetric matrix suggests that a model favoring fusions may be more appropriate. When

the ratio of cost of fissions to fusions is simply assumed to be the mean ratio estimated from equal costs (i.e., 2.18:1), the single most parsimonious reconstruction requires 30 steps, of which only one is a fission ($2n = 38$ to $2n = 40$). When we increase the relative cost of fissions to 3:1, there are two equally parsimonious reconstructions, each requiring 31 steps; one of the reconstructions requires a single fission, and the second involves none. Thus if fissions are three times less likely than fusions, our data may be explained entirely in terms of fusions. When the cost ratio increases beyond 3:1, it is always most parsimonious to reconstruct all rearrangements as fusions. Note that a ratio of fusions to fissions of at least 3:1 is not only biologically plausible, it is well within the range estimated with a symmetrical step matrix. Step matrix analysis is consistent with our assumption that rearrangements in *Planipapillus* consist exclusively of fusions.

A second approach to the analysis of step matrices involves estimation of the phylogenetic signal contained in the karyotype data when different matrices are assumed. The logic is as follows. Parsimony tree length is a function of the observed data, the hypothesized tree topology, and the model of character evolution (i.e., the step matrix). If the observed data are the product of evolution, they will come from a probability distribution determined by true tree topology and true character evolution model. This nonindependence makes the length of the true tree under the true model deviate from that expected for a random tree or random model. Thus, just as we identify a parsimonious tree topology as one that has a shorter-than-expected length, we can identify a character evolution model by the same criterion. The distribution of expected tree lengths can be generated by randomization. Randomization tests for phylogenetic signal have been described in other contexts (e.g., Archie 1989; Faith 1991; Hillis and Huelsenbeck 1992; Ree and Donoghue 1998). For each of five character evolution models, we have generated two sets of null distributions: (1) tree lengths for random topologies; and (2) tree lengths for permutations of the data. We can then see which matrix maximizes the fit between the matrix, the assumed true topology, and the observed data, where fit is measured as the deviation of tree length from the expectation for random topologies and random data.

Figure 2 shows that the deviation from the null expectation is maximized when the character evolution model disallows fissions. The significance of the fit between the observed data, the assumed topology, and the model of character change increases monotonically as the relative costs of fissions and fusions shifts from fusions disallowed through equal probability of fusions and fissions to fissions disallowed. Although the symmetric model requires fewer rearrangements (27 vs. 31), the fusions-only model shows the strongest evidence of nonrandomness, that is, phylogenetic signal. Given the results of the two approaches above, we adopt the fusions-only model for subsequent analyses.

Assignments of Fusions to Branches

Under the parsimony criterion, there is a single best reconstruction with 31 fusions, shown with diploid numbers assigned to nodes in Figure 3. The maximum-likelihood karyotype reconstructions are also indicated in Figure 3. Under the unconstrained (24-rate) model, the maximum-likelihood reconstruction requires 32 fusions ($-\ln L = 15.558083$). The reconstruction with the highest likelihood under the one-rate Poisson model requires 37 fusions ($-\ln L = 46.509941$). The two-log-likelihood unit confidence intervals for these reconstructions are quite large, including 0.9% of all possible reconstructions in the one-rate case and 4.2% in the 24-rate case. Figure 4 shows the confidence intervals in terms of the number of fusions for each reconstruction. The maximum-parsimony reconstruction is only slightly less likely than the maximum-likelihood reconstruction under the 24-rate model ($-\ln L = 16.114277$). Under the one-rate model, the parsimony reconstruction is rejected ($-\ln L = 52.286871$).

Testing Models of Karyotype Evolution

We have tested the single-rate Poisson model by calculating the likelihood-ratio test statistic, δ. The statistic sums over all possible reconstructions of ancestral states and the test are thus independent of the accuracy of the point estimates of ancestral states. δ is 65.531554 with a 95% confidence interval, estimated by bootstrap method, ranging from 60 to 75. When δ is calculated for each reconstruction individually, the lowest observed value is 55. The values for the parsimony reconstruction and the one- and 24-rate maximum-likelihood reconstructions are 72, 56, and 73, respectively.

The null distribution for δ should asymptotically approximate the χ^2 distribution with 23 degrees of freedom, which yields high significance, $P < 10^{-5}$. We have also simulated the null distribution at six rates as described in Materials and Methods. The rates considered are representative of the range implied by the possible karyotype reconstructions. The distributions for the six rates are very similar, as shown by representative curves in Figure 5, although the nonparametric Kruskal-Wallis H test indicates that they differ significantly ($P = 0.01$). They all differ from the χ^2 distribution (df = 23). The critical fact is that in none of the 6000 simulations was δ as high as observed for the *Planipapillus* data; the highest value observed in simulation is 53.2. Whether the simulations or χ^2 distributions are considered, the constant-rate Poisson model for *Planipapillus* karyotype evolution is rejected at the $P < 0.001$ level.

Discussion

Karyotype Reconstruction

Our phylogenetic approaches support our a priori assumption (Rowell et al. 2002) that Robertsonian rearrangements in *Planipapillus* are centric fusions. Given the fusions-only model, parsimony and likelihood methods assign different ancestral states to several nodes in the phylogeny, but most nodes are reconstructed identically by each of the methods (Fig. 3). The one-rate maximum-likelihood reconstruction requires many more fusions than the other reconstructions, however. The 24-rate reconstruction differs from the parsimony reconstruction at only a single node, which is descended from the shortest branch in the phylogeny. These results show that character reconstruction is sensitive to the model

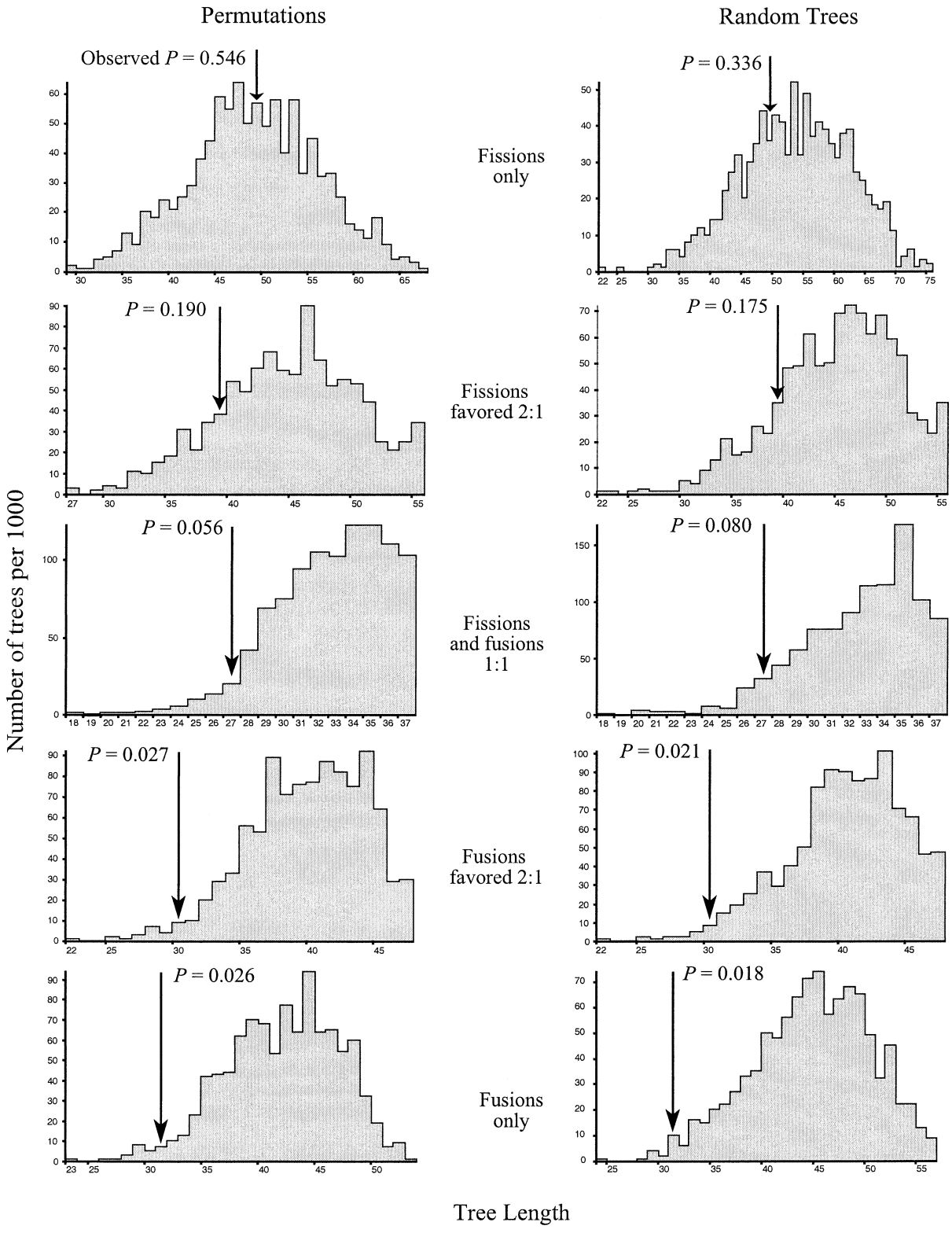

FIG. 2. Distributions of tree lengths for permutations of the observed karyotype data (left) and random trees (right) under five models of Robertsonian change. The arrows indicate the values for the observed data and tree.

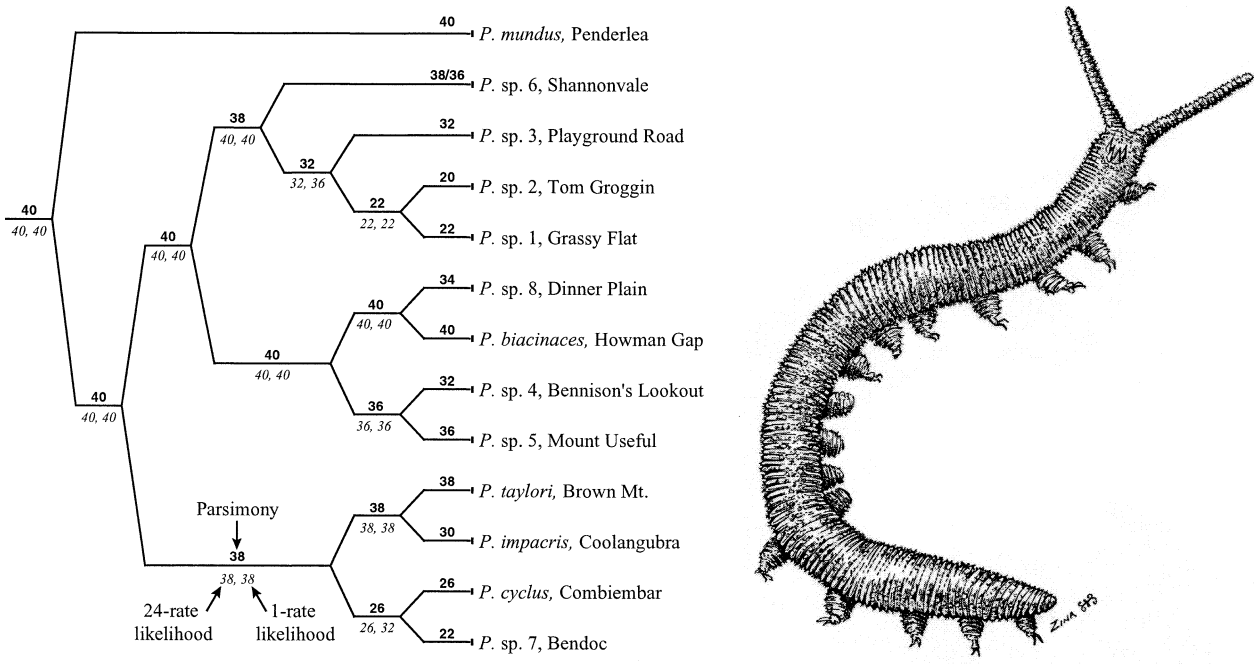

FIG. 3. Reconstruction of ancestral karyotypes. Each internal branch records the diploid number reconstructed for the node to its right. In bold, above the branch, is the parsimony reconstruction. In italics, below the branch, are maximum-likelihood reconstructions, first for the 24-rate model, then for the one-rate model. At right, a male *Planipapillus impacris* from Coolangubra, New South Wales.

assumptions of the method, whether implicit (parsimony) or explicit (likelihood).

Rejecting the Constant-Rate Neutral Rearrangement Model

We have tested the null hypothesis that karyotype evolution in *Planipapillus* follows a constant-rate Poisson process. We have adapted this hypothesis from studies of molecular evolution, in which the constant-rate Poisson process is held to be a model of neutral evolution with a constant rate of neutral mutation (e.g., Ohta and Kimura 1971; Langley and Fitch 1974; Kimura 1983; Takahata 1987; Gillespie 1989, 1991; Zeng et al. 1998; Cutler 2000). For the karyotype data, the analogous process would imply that each chromosome rearrangement is neutral and the rearrangement rate constant. Many studies have proposed that centric fusions and other classes of rearrangement may be neutral, and neutrality is an assumption of some models of speciation by centric fusions with monobrachial homology. Our test therefore evaluates and rejects a submodel of these neutral rearrangement models in which the mutation rate is constant.

Caveats

Coalescence

Rejection of the constant-rate Poisson process is not strictly equivalent to rejection of the neutral-rearrangement, constant mutation model. Gillespie and Langley (1979) and Hudson (1983) showed that under the constant mutation-rate neutral model, deviations from the Poisson process will result from variances introduced by the coalescent process. This pattern is due to the difference between the time to the common ancestor of two populations, t_i, and the time to the ancestor of the two alleles sampled from them, T_i. The distribution of the number of rearrangements since T_i is a function of t_i and $\theta = 4N\mu$, where N is the population size and μ the mutation rate. Because populations with large θ will have high polymorphism, samples from these populations will have the greatest difference between t_i and T_i and so will introduce the largest deviation from Poisson. Any deviation from the Poisson expectation of the likelihood-ratio test statistic can be explained under the neutral process by a sufficiently large θ (Hudson 1983).

In the present case, we consider the rejection of the Poisson process to constitute rejection of the constant-rate neutral rearrangement model despite the complication introduced by the coalescent process. First, θ is likely to be quite small for *Planipapillus* chromosomes, because Australian onychophorans live at low densities and appear to have very small populations (New 1995; Briscoe and Tait 1995). Small θ is supported by the observation that mitochondrial haplotype diversity in *Planipapillus* is exceptionally low, much lower than the levels of divergence among even the most closely related species (Rockman et al. 2001). Second, because we estimate times t_i from gene sequences, that is, alleles drawn from the populations, our $\hat{t}_i$ are weighted averages of $\hat{T}_{i,ftz}$ and $\hat{T}_{i,mt}$, the times to the common ancestors of the nuclear *ftz* intron alleles and the mitochondrial genomes of the sampled populations. We have established by likelihood-ratio test that the distributions of substitutions in these sequences are consistent with a constant-rate Poisson process, the molecular clock. Consequently, our $\hat{t}_i$ are really $\hat{T}_{i,sequence}$. The amount of time between t_i and T_i, which is the source of deviations from Poisson under the neutral model, is, for our data, given by the difference between $\hat{T}_{i,sequence}$ and the unknown

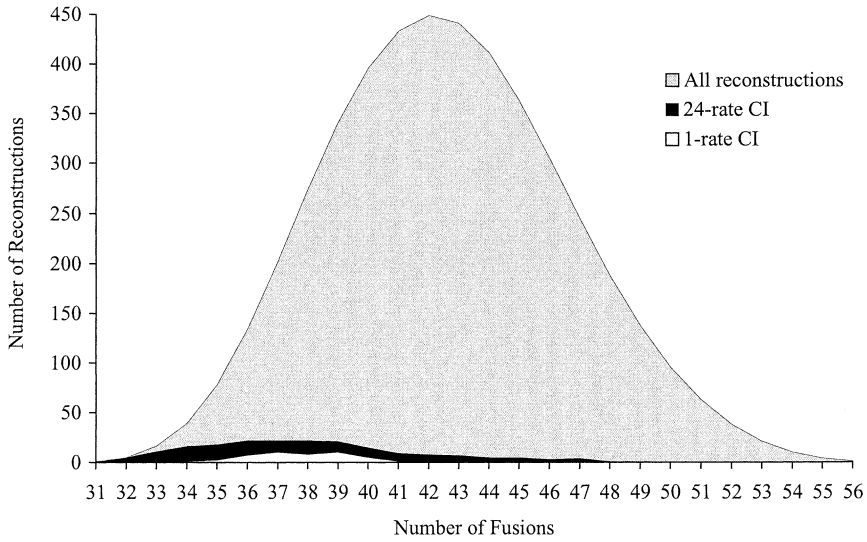

FIG. 4. Distribution of the number of all possible reconstructions requiring a given number of centric fusions. Superimposed on this distribution are the distributions of the reconstructions included in the 2-log-likelihood unit confidence intervals (CI) for the one- and 24-rate maximum-likelihood reconstructions.

$T_{i,karyotype}$; under neutrality this difference is likely to be smaller than the amount of time between $T_{i,karyotype}$ and t_i, the time of isolation of the populations. The neutral rearrangement, constant mutation model corresponds to the Poisson model precisely when the mean number of rearrangements since time $T_{i,karyotype}$ is proportional to that time. Under the assumption of neutrality our estimates of lineage branching times are unlikely to differ much from the $T_{i,karyotype}$. We have also shown by bootstrapping that the rejection of the single-rate Poisson is insensitive to the precise branch lengths. We do not think that the coalescent process can explain the observed deviation from the Poisson process; nonneutrality and/or variation in mutation rate must be invoked.

Lineage effects

Gillespie (1989, 1991) has drawn a useful distinction between two classes of deviation from constant-rate Poisson processes on evolutionary trees: Rates may be Poisson distributed with a changing mean rate (lineage effects), or rates may have a constant mean but not be Poisson distributed (residual effects). Lineage effects include rate variation due to variation in organismal characteristic such as generation time and metabolic rate. If rearrangements are Poisson distributed per generation or as a function of metabolic rate, but generation time or metabolic rate vary among the lineages, then the number of rearrangements in absolute time will deviate from the constant-rate Poisson expectation. Residual

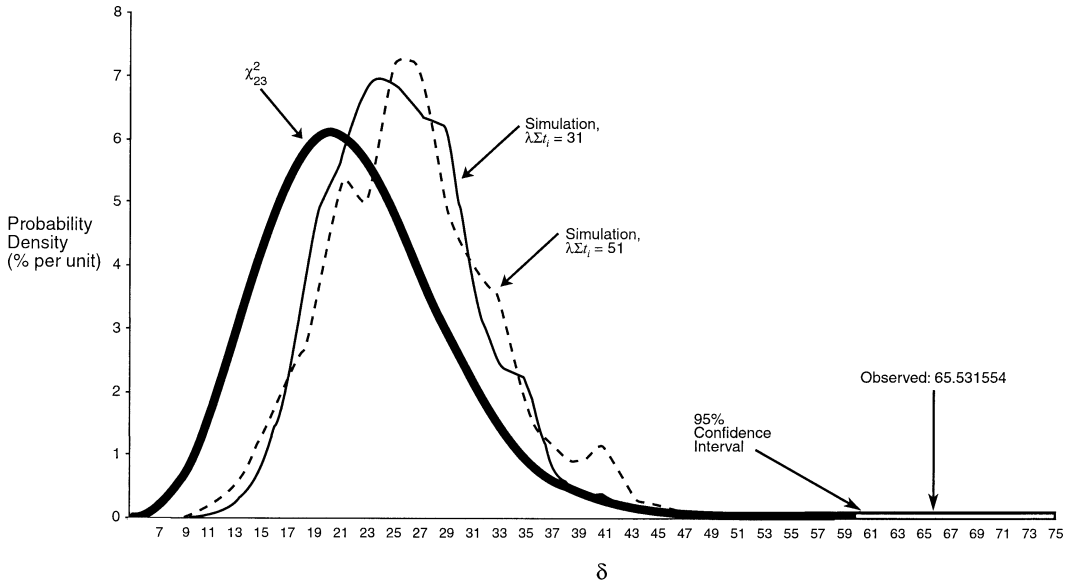

FIG. 5. Distributions of the simulated likelihood ratio test statistic δ and the χ^2 distribution with 23 degrees of freedom.

effects include the influences of selection and non-Poisson mutational processes and are our primary interest.

Lineage effects are unlikely to contribute substantially to the observed evolutionary rate variation in *Planipapillus*. First, the species are very closely related. Although little is known about the basic biology of *Planipapillus*, it seems unlikely that the studied populations differ significantly among themselves in generation time or metabolic rate. This contrasts with the rate comparisons often made in the literature, which feature taxa separated by tens of millions of years and known to have significantly different life histories and physiologies (e.g., mammals: Gillespie 1989; Ohta 1995; and *Drosophila*: Takano 1998; Zeng et al. 1998).

Second, if lineage effects are influencing rates of karyotype evolution, a similar effect should be observed for rates of nucleotide sequence evolution. Instead, as described above, the concatenated nucleotide sequences are compatible with a molecular clock. We can also test the Poisson model of karyotype evolution with an internal control on lineage effects by using branch lengths estimated from the nucleotide data without the molecular clock enforced. We ask whether the amount of karyotype evolution is correlated with the amount of nucleotide evolution. This approach is comparable to the genetic model for continuous characters of Mooers et al. (1999) and to Gillespie's (1989) branch-weighting method for sequence data. We calculated the likelihoods for each possible karyotype reconstruction with the nonclock branch lengths, under both the one-rate model and the unconstrained (24-rate) model; δ is 63.61048, slightly less than under the strict clock model, but still significantly different from the one-rate Poisson expectation.

Our data differ from most sequence data in that there are a finite number of possible rearrangements due to the irreversibility (fusions-only) assumption. Thus, one could envision a lineage-effect scenario in which rearrangement rate is a function of the number of possible fusions, for example, the number of telocentric chromosomes (i.e., $2n - 20$). This scenario is not supported by the data, however. The rate on each branch is not correlated with the number of telocentrics at the start of the branch (24-rate maximum-likelihood reconstruction; $r = -0.08$, not significantly different from zero according to a two-tailed *t*-test, $P = 0.70$). Although in the long run *Planipapillus* rearrangements may become saturated, as all the populations exhaust the possible fusions, at present only one population has reached that limit. The high rate variation in *Planipapillus* karyotype evolution cannot be explained by a gradual slowing of the mutation rate.

Framing and Testing Alternative Models

Having rejected the neutral-rearrangement, constant mutation rate model for *Planipapillus* karyotypes, we now consider other models and means to test them. An important consequence of rejecting the constant-rate neutral model is that rate variation in *Planipapillus* should have a cause, and we may seek correlations between lineage-specific rates and lineage-specific demographic and biological characteristics. We consider three possibilities, that the centric fusions are (1) favored by selection under some circumstances; (2) neutral but mutation rates vary; and (3) underdominant. These are not mutually exclusive explanations.

Chromosomal rearrangements, even in the absence of genic changes, can be advantageous. Centric fusions are capable of dramatically altering the spatial distribution of chiasmata within chromosome arms, and thus of changing recombination patterns and producing novel multilocus genotypes (Bidau 1990; Rowell 1991). This phenomenon seems unlikely to be responsible for the fixation of *Planipapillus* fusions, however, because fusions are systemic throughout the karyotype, involving every chromosome arm, and each fusion would have to produce advantageous new genotypes in each population—minimally 31 times in the clade. Chromosome rearrangements may produce systemic advantage, by influencing the stability of the nucleus (Imai et al. 1994) or by changing the duration of the cell cycle in such a way as to alter developmental time (Groeters and Shaw 1992). If the advantage of centric fusions is systemic, positive selection would act on the diploid number rather than on fusions of particular chromosomes. Such a model of systematic advantage makes two predictions: diploid numbers should be associated with some sort of environmental variable, and variation should be clinal, at least locally, such that intermediate diploid numbers and heterozygotes should occur geographically between extreme numbers. Although we have not yet parsed all possible relationships between environmental variables and diploid number in *Planipapillus*, a strong relationship seems unlikely. For example, two species whose karyotypes differ the most—*P. mundus* at Penderlea ($2n = 40$) and *P.* sp. 2 at Tom Groggin ($2n = 20$)—are geographically very close to one another and experience similar climates and other habitat conditions. In addition, the absence of heterozygotes or forms with intermediate chromosome numbers in the immediate region argues against a role for positive selection on diploid number.

A role for positive selection may also be invoked at genic loci linked to neutral chromosome rearrangements. Individual neutral rearrangements may be fixed due to hitchhiking with favorable genic mutations, particularly if fusions move crossovers to the distal ends of the chromosomes, minimizing recombination (Bidau 1990; Rowell 1991). However, in the absence of a systematic association between centric fusions and favorable genic mutations, hitchhiking will not cause the fixation of neutral fusions to deviate from a Poisson process (Birky and Walsh 1988).

If centric fusions in *Planipapillus* are effectively neutral, the spontaneous mutation rate must vary among lineages. Such variation is well supported for centric fusions in *Mus domesticus*; in that taxon, the so-called Robertsonian races, which possess metacentric chromosomes, show high rates of spontaneous fusion among the telocentric chromosomes, whereas the wild-type, all-telocentric races show low rates of spontaneous fusion (Redi et al. 1990; Nachman and Searle 1995). Moreover, although fusions are common in *M. domesticus*, they are virtually absent from the closely related *M. musculus* (Nachman and Searle 1995). A heritable tendency to spontaneous centric fusion has also been demonstrated in the grasshopper *Atractomorpha similis* (Peters 1982). In spiders, centric fusion is largely an all-or-none phenomenon: If any autosomes are involved in fusions, all

are (Rowell 1990). There thus appear to be systematic biases in chromosomal mutation rates and spectra in different lineages.

It is now well established that chromosome rearrangements, including centric fusions, may be caused by ectopic recombination between transposons, with transposition bursts causing high levels of chromosome rearrangement (Redi et al. 1990; Lim and Simmons 1994; McDonald 1995; Waugh O'Neill et al. 1998; Cáceres et al. 1999). Variation in rearrangement rate among lineages would then correlate with variation in the transposition rate, which in turn is correlated with small population size, inbreeding, and genomic stress (Peters 1982; Fontedevila 1992; Lim and Simmons 1994; McDonald 1995; Waugh O'Neill et al. 1998; Capy et al. 2000). An explanation of *Planipapillus* chromosome evolution relying on mutation rate variation therefore makes predictions about variation in population biology among *Planipapillus* species; in particular, a role for transposition implies a negative association between rates of chromosome evolution and effective population size.

The third selection regime we consider is underdominance, which is the classic model for most chromosome rearrangements. All models for the fixation of underdominant rearrangements require drift and thus predict strong associations with effective population size, via small census sizes, high inbreeding, strong demic structure, and high rates of population founding (Wright 1941; Lande 1979, 1985; Hedrick 1981; Hedrick and Levin 1984; Sites and Moritz 1987; Coyne 1989; Barton and Rouhani 1991; Spirito 1992; Michalakis and Olivieri 1993). *Planipapillus* population structure is likely to include all of these elements. If *Planipapillus* rearrangements are underdominant, we predict a relationship between diploid number and population size, roughly with the highest historical population sizes found for *P. mundus* and *P. biacinaces* ($2n = 40$) and the lowest for *P.* sp. 2, Tom Groggin ($2n = 20$).

In summary, our reconstruction of karyotype evolution in *Planipapillus* limits the possible roles played by selection, mutation, and drift. Our discussion suggests that variation in rearrangement rates, underdominance, or both should be invoked to explain the karyotype data. In either case we predict a negative relationship between rearrangement rate and historical population size.

Implications for Speciation in Planipapillus

Figure 3 shows that almost every splitting event in *Planipapillus* phylogeny is associated with a change in diploid number. In the parsimony reconstruction, there is one exception, the most basal split in the tree, whereas the likelihood reconstructions imply two exceptions. Although chromosome rearrangements may play no role in *Planipapillus* speciation, the close association between branching events and fusions raises the possibility that the rearrangements contribute to reproductive isolation among *Planipapillus* populations.

Speciation by centric fusions with monobrachial homology (Capanna 1982; Baker and Bickham 1986) is analogous to the Dobzhansky-Muller model (Orr 1995, 1996; Coyne et al. 1997) for speciation by complementary gene interactions (more recently called the synthetic model: Coyne et al. 2000) and to models of speciation on holey landscapes (Gavrilets 1999). In each of these models, individual mutations may be fixed selectively or neutrally, whereas in the higher dimensionality of multilocus fitness landscapes the intermediates are selected against. The monobrachial homology model differs from the Dobzhansky-Muller model in one important respect, however, in that fusions must be fixed in each lineage; unlike the case for genes, sequential fixations of fusions in only one lineage cannot create isolation.

Reproductive isolation in the centric fusion model emerges from meiotic interactions between chromosomes with monobrachial (single-arm) homology. Although we cannot assign identities to individual *Planipapillus* chromosome arms, our reconstructions imply that some geographically close populations have independently derived metacentrics. For example, *P. impacris* and *P.* sp. 7, species from localities near to one another in the temperate rainforests along the eastern Victoria–New South Wales border, have accumulated four and eight fusions independently since their common ancestor, according to our reconstructions. These karyotypes are almost certain to include chromosomes with monobrachial homologies. However, most sister taxa in our reconstruction do not permit the possibility of monobrachial homologies because only one of the two daughter lineages is inferred to have fusions. In these cases neutral fusions cannot play a role in reproductive isolation. In addition, the failure of fusions to have introgressed into the all-telocentric populations cannot be explained by chromosomal speciation if individual rearrangements are neutral.

Rieseberg's (2001) speciation model explains the absence of introgression by positing the evolution of isolation genes linked to the rearrangements. However, the rate of fixation of fusions in *Planipapillus* is so high—more than one fusion for every 0.001 nucleotide substitutions per site across the whole clade—that positing the rapid evolution of isolation genes linked to each one seems both implausible and ad hoc.

If rearrangements are weakly underdominant, each individual fusion contributes to isolation and multiple fusions may cumulatively create effective reproductive barriers (Walsh 1982; Searle and Wójcik 1998; Castiglia and Capanna 2000). In *Planipapillus,* sister species differ by at least two fusions, with a single exception. Thus we need not hypothesize dramatic underdominance for *Planipapillus* fusions to contribute to speciation. A more reasonable interpretation assigns each fusion weak underdominance; these rearrangements are fixed by drift, in a population-size dependent manner akin to the nearly neutral variants of molecular evolution models, and cumulatively create reproductive isolation. Unlike neutral models, this scenario explains the persistence of $2n = 40$ karyotypes.

Phylogenetic methods represent a promising addition to the toolkit for studying the dynamics of chromosome evolution. Our proposal that *Planipapillus* rearrangements are either underdominant or subject to episodic mutation creates a framework for future investigations, using other methods, into the evolution of chromosomes and their role in the radiation of the clade.

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