Dispersal Capacity Predicts Both Population Genetic Structure and Species Richness in Reef Fishes

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abstract: Dispersal is a fundamental species characteristic that should directly affect both rates of gene flow among spatially distributed populations and opportunities for speciation. Yet no single trait associated with dispersal has been demonstrated to affect both micro- and macroevolutionary patterns of diversity across a diverse biological assemblage. Here, we examine patterns of genetic differentiation and species richness in reef fishes, an assemblage of over 7,000 species comprising approximately one-third of the extant bony fishes and over one-tenth of living vertebrates. In reef fishes, dispersal occurs primarily during a planktonic larval stage. There are two major reproductive and parental investment syndromes among reef fishes, and the differences between them have implications for dispersal: (1) benthic guarding fishes lay negatively buoyant eggs, typically guarded by the male parent, and from these eggs hatch large, strongly swimming larvae; in contrast, (2) pelagic spawning fishes release small floating eggs directly into the water column, which drift unprotected before small weakly swimming larvae hatch. Using phylogenetic comparative methods, we show that benthic guarders have significantly greater population structure than pelagic spawners and additionally that taxonomic families of benthic guarders are more species rich than families of pelagic spawners. Our findings provide a compelling case for the continuity between micro- and macroevolutionary processes of biological diversification and underscore the importance of dispersal-related traits in influencing the mode and tempo of evolution.

Keywords: dispersal, diversification, key innovation, life-history traits, planktonic larvae, population genetic structure, species richness.

Introduction

Reductions or interruptions in gene flow are central to the divergence of populations (Wright 1931; Slatkin 1987) and

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species (Dobzhansky 1940; Mayr 1963; Coyne and Orr 2004; Marie Curie Speciation Network 2012). However, identifying key life-history traits (Heard and Hauser 1995) affecting gene flow and demonstrating a statistical effect on within- or between-species diversity is challenging. Relevant traits are difficult to categorize or quantify, statistical tests require evolutionary replication among lineages, species-specific information is required for many species across lineages, and the inherent complexities and stochasticity of biological systems are likely to mute the signal of any effect.

Theoretically, dispersal and genetic differentiation should be inversely related (Wright 1931; Slatkin 1987). However, the degree to which an organism's dispersal capacity affects population genetic structure is an open question, with support varying among taxa and methodological approaches (reviews by Bohanok 1999; Givnish 2010). Although outcomes are inconsistent across studies, specific dispersal traits that have been tested extensively for their influence on intraspecific genetic differentiation include seed type and pollination mode in plants (Loveless and Hamrick 1984; Hamrick and Godt 1996; Duminil et al. 2007; Givnish 2010), pelagic larval duration among marine invertebrates and fishes (Bradbury et al. 2008; Kelly and Palumbi 2010; Riginos et al. 2011; Selkoe and Toonen 2011; Dawson et al., forthcoming), and habitat usage in rainforest birds (Burney and Brumfield 2009). Undoubtedly, observed population genetic structure of any species is influenced by a myriad of factors, including sampling, the spatial complexity of habitat, species range size, and recent evolutionary history; any of these factors may obscure patterns of intraspecific genetic differentiation resulting from specific dispersal-related traits.

Predictions about the relationship between dispersal and species diversification are contradictory: for example, re-

duced dispersal could enhance opportunities for speciation (Mayr 1963); alternatively, species with greater range sizes (and presumably greater dispersal ability) will encounter more potential barriers to dispersal and therefore should have greater opportunities for speciation (Rosenzweig 1995; reviewed by Jablonski and Roy 2003) and reduced likelihood of extinction (Gaston and Chown 1999). Others suggest that intermediate dispersal abilities would maximize diversification by allowing rare long-distance colonization but not enough gene flow to swamp genetic divergence (Gaston and Chown 1999; Paulay and Meyer 2006; Claramunt et al. 2012).

Although many studies have examined the influence of key traits on macroevolutionary patterns, there have been relatively few attempts to correlate dispersal-related traits with species diversification. For angiosperms, the influence of animal-vectored dispersal of seeds has been investigated. Animal pollination is significantly associated with diversification (Eriksson and Bremer 1992; Dodd et al. 1999; reviewed by Givnish 2010); however, mutualisms between animal pollinators and plants would also promote plant speciation by isolating gene pools, and thus, the effects of mutualisms are difficult to distinguish from effects via pollen dispersal. Whether animal dispersal of seeds, particularly those with fleshy fruits, has contributed to angiosperm diversification by reducing dispersal appears dependent on habitat and taxon (Givnish 2010); for instance, greater species richness was found among tropical understory plants with fleshy fruits (Smith 2001), woody (but not herbaceous) plants with biotic dispersal (Tiffney and Mazer 1995; Bolmgren and Eriksson 2005), and taxa where ants disperse seeds (Lengyel et al. 2009). An additional consideration is that extant patterns reflect both extinction and speciation: in the case of woody plants, fleshy fruits might increase the reliability of transport to suitable locations in unpredictable habitats and thus lower extinction risk (Tiffney and Mazer 1995; Bolmgren and Eriksson 2005). Clearly among plants there is no simple diversification outcome arising from dispersal mode alone.

The few empirical studies examining the relationship between dispersal capacity and diversification in animals have focused on birds, and the results have been mixed. Dispersal ability was inversely proportional to subspecies richness in British birds (Belliure et al. 2000) and rates of diversification were lower for more dispersive lineages of Furnariid birds (Claramunt et al. 2012) consistent with the hypothesis that low dispersal enhances speciation probability. In contrast, more dispersive bird families were found to be more species rich than their less dispersive sister families (Owens et al. 1999) and had higher diversification rates (Phillimore et al. 2006). Thus, the effects of dispersal traits on animal diversification are unclear but not cohesively investigated for most animal taxa.

Diversification is the net result of speciation and extinction, processes that occur at the species and population level. Empirical measurements of genetic differentiation, therefore, could provide a more direct link to diversification than dispersal related. Moreover, population genetic structure should capture contributions both from dispersal and habitat structure (spatial and environmental) and therefore might be a superior predictor of speciation (or extinction) probability. Yet the relationship between within-species genetic differentiation and macroevolutionary patterns is virtually unknown. In a rare exception, Kisel and Barraclough (2010) explicitly examined this relationship and found that average levels of population genetic structure (among broad categories of disparate terrestrial taxa) were good predictors for speciation rates on oceanic islands. No specific biological trait, however, was investigated as a cause for this pattern. Indeed, the paraphyletic assemblage considered (snails, angiosperms, birds, lizards, bats, etc.) does not share obvious sets of traits affecting dispersal or population genetic structure. The predicted genetic differentiation–diversification association was also tested in a monophyletic assemblage of orchids (Kisel et al. 2012), whereby five sister clades of differing species richness were targeted and genetic differentiation from one to three exemplar species within each sister clade was contrasted. Contrary to expectations, no difference was found in levels of genetic differentiation between clades of high and low species richness. Finally, Burney and Brumfield (2009) considered habitat usage as a dispersal-related trait and suggested that rain forest birds foraging in tree canopies were more generalist in habitat preference than understory birds and therefore dispersal should be greater among canopy birds. Consistent with their prediction, there was significantly less genetic differentiation among canopy-using Amazonian birds as contrasted with codistributed understory birds. Additionally, subspecies diversity among canopy birds worldwide was greater than for understory birds. In summary, although some individual studies identify links among combinations of dispersal traits, genetic differentiation, and macroevolution, uniform and compelling associations are not evident.

Like genetic differentiation, range size is an emergent species trait indicative of dispersal (Jablonski 2008). The connection between dispersal, range size, and diversification has been most thoroughly investigated for Cretaceous and Tertiary marine molluscs, providing the only example we can find in the literature where the effect of a dispersal trait on both an emergent species-level trait and macroevolutionary patterns has been simultaneously evaluated. Gastropods with feeding planktonic larvae had greater range sizes compared to species with nonfeeding

larvae (Hansen 1980; Jablonski 1986; Jablonski and Hunt 2006) although this relationship was not found for bivalves. In addition, gastropods with larger ranges had longer species durations (Hansen 1980; Jablonski 1987; Jablonski and Hunt 2006). Taking the three levels of cause and effect together (i.e., dispersal trait, emergent trait, and macroevolutionary pattern), Jablonski and Hunt (2006) demonstrated that range size was a sufficient predictor of gastropod species duration with negligible additional signal provided by larval feeding mode, suggesting that feeding mode alone was not the key driver of macroevolutionary patterns.

Detection of the hypothesized relationship between dispersal traits, population genetic structure, and diversification requires strong mechanisms that are moderately evolutionarily conserved to yield discernible patterns of biological diversity. In actuality, (i) dispersal capacity arises as an aggregate of various biological traits and habitat structure, (ii) population genetic structure is not a straightforward reflection of time averaged dispersal (Whitlock and McCauley 1999), (iii) dispersal affecting traits may not be phylogenetically conserved, (iv) high variance in population genetic structure among species is likely such that lineage estimates based on one or a few species are probably not representative of the lineage average, and (v) extant species richness is the end product of both speciation and extinction, processes that will be affected by many abiotic and biotic factors (Coyne and Orr 2004). Thus, even if traits genuinely influence population genetic structure and speciation statistical associations may be undetectable in most natural systems.

In this study, we investigate the relationships among dispersal-related traits, population genetic structure, and patterns of diversification in marine reef fishes. Reef fishes are well suited for testing evolutionary hypotheses related to dispersal: adults are fairly sedentary and dispersal occurs primarily via eggs and planktonic larvae. Most reef fishes either release pelagic eggs directly into the water column, from which hatch weakly swimming larvae, or guard benthic (or brooded) eggs that hatch strong swimming larvae. These two reproductive strategies are widely distributed across taxonomic groups of fishes but consistent within taxonomic families (Thresher 1984; Leis 1991; Cowen and Sponaugle 1997). Larvae from guarded benthic eggs generally have a shorter pelagic duration and are more likely to complete development inshore, thus their average dispersal distance is presumably less than the dispersal distance of larvae hatched from pelagic eggs (Leis and Miller 1976; Leis et al. 1998; Leis et al. 2003). Here, we test the hypotheses that benthic guarders have greater population genetic structure and more opportunities for speciation as compared to pelagic spawners.

Methods

Focal Taxa

We only considered taxonomic families of fishes with demersal (living on the bottom: not pelagic) adults of primary marine habit (Nelson 1994), whose reproductive strategies are well known. Families were categorized as "benthic guarders" or "pelagic spawners" (Thresher 1984; Leis 1991; Cowen and Sponaugle 1997). Here, benthic guarders include families that brood their young (Syngnathidae and some Apogonidae) or lay benthic eggs, typically followed by paternal guarding of unhatched eggs. Pelagic spawners release floating eggs and perform no parental care. Antenniirids are the only reef family known to include species that guard nests or young and also release pelagic eggs (albeit in floating chains). Because these traits are variable and poorly studied in Antenniirids (see Arnold 2010 for a comprehensive review), we excluded the Antennariidae from our analyses. Fishes that scatter their eggs (benthic eggs, but no parental care: Balistidae, Monacanthidae, Tetraodontidae, and Siganidae) were provisionally grouped with pelagic spawners. Sebastids whose larval characteristics are similar to larvae from pelagic eggs (small size and long larval duration) despite being viviparous were also provisionally grouped with pelagic spawners. Scatterers and Sebastids were included in the reported full linear models, although excluding these taxa did not affect the significance of the results. Families were assumed to be monophyletic, with the exception of the Serranidae, which were split into several monophyletic groups (as suggested by Smith and Craig 2007), and the Labrids, which were combined with the Scarids (following Westneat 1993). Species richness of each family was based on number of valid species names (Eschmeyer and Fong 2009).

Population Genetic Structure

Estimates of population genetic structure were taken from Riginos et al.'s (2011) compilation of reported estimates for marine fishes. Species from families without strong reef associations or from families that contained some freshwater or brackish water species were removed from our data set as were data based on restriction enzyme fragment patterns: this was the genetic category with the fewest species records, and only seven species records remained once species from families with freshwater associations were excluded. In addition, the low number of species with restriction fragment type data precluded parameter estimation for the genetic marker-by-geographic extent interaction term in multivariate models (analyses by Riginos et al. 2011 had identified the importance of the genetic marker-by-geographic extent interaction term in such models). In instances where there were multiple studies

of the same species (for example, two studies employing different genetic markers), a single species datum was retained based on prioritizing studies using mtDNA sequences over microsatellites or allozymes. If there was a choice within marker type, data from the study that surveyed the greatest geographic extent were retained. Prioritizing data inclusion by greatest geographic extent over marker type made no difference to the overall results and only affected data for two species.

Phylogenetics

There is no complete phylogenetic tree including all the focal families considered in this study, much less the focal species. To create a genealogical hypothesis that would permit us to account for phylogenetic correlations, rhodopsin (*Rhod*) sequences (from Li and Nei 1975; Li et al. 2009) were parsed to retain focal reef fish families and to preserve fossil-dated calibration points (from Santini et al. 2009; nodes 24, 33, 34, 36, 37, 38, 42, and 44 from their table 2) and augmented by additional *Rhod* sequences representing focal families as available from the National Center for Biotechnology Information (NCBI). NCBI and the Barcode of Life Database (BOLD) were queried for cytochrome oxidase I (*COI*) sequences for all species for which we had an estimate of population genetic structure and for each species with a *Rhod* sequence. *Rhod* and *COI* sequences were concatenated with missing loci treated as missing data. A Bayesian search was conducted in MrBayes (ver. 3.2, Ronquist and Huelsenbeck 2003), with four partitions representing the two genes and treating third positions separately from first and second positions for each gene. Trees were constructed in two manners to probe the sensitivity of results to tree shape. First, taxonomic families and clades receiving ≥90% support on a recent and well-resolved acanthomorph phylogeny (Near et al. 2013) were constrained to be monophyletic in our search. In the second tree search, only taxonomic families and the Percomorpha were constrained to be monophyletic. Searches were conducted using 10 million steps (sufficient for convergence in the two chains: SD < 0.01) and a burn-in of 2.5 million steps under a GTR + G + I model of evolution for each partition. The resultant consensus trees were transformed to chronograms using penalized likelihood with the truncated Newton method (r8s, ver. 1.7; Sanderson 2002) using fossil calibration points (Santini et al. 2009), and these chronograms formed the basis of the subsequent phylogenetic analyses. To estimate the depth of nodes that define taxonomic families, we took all public *COI* sequences from BOLD (October 2012) from each family, aligned (Larkin et al. 2007) and trimmed sequences to the same 650-basepair region, and then manually inspected each alignment alongside a neighbor-joining tree based on that alignment to remove poor quality or suspected pseudogene sequences. We then took the maximum pairwise distance (Tamura-Nei distance in DNAdist of the PHYLIP 3.69 package; Felsenstein 1993) as an indicator of relative clade depth. Nexus files have been deposited with TreeBASE.

Reproductive Strategy as a Predictor of Population Genetic Structure and Species Richness

We predicted that benthic guarding species should have greater population genetic structure than pelagic spawning species; however, estimates of population genetic structure might also be affected by the genetic marker used and the geographic distance surveyed. Thus, our predictors include both categorical (reproductive strategy and genetic marker) and continuous (geographic extent) variables. Therefore, we first used ordinary (general) linear models (ordinary least squares [OLS]) to test our expectations, where genetic marker, geographic extent, and reproductive strategy were independent predictors of population structure by species. The response variable was the natural logarithm of Rousset's (1997) transformation of species F_{ST} (see Riginos et al. 2011 for further details); this transformation resulted in residuals that conformed to the normality assumption.

The most common approach for evaluating the effects of multiple independent variables on Gaussian response variables (such as F_{ST} or species richness) in a phylogenetic framework is phylogenetic generalized least squares regression (PGLS; Grafen 1989). In PGLS, the nonindependence of trait values across taxa, induced by their shared evolutionary history, is accounted for by applying an estimate of phylogenetic covariance to the response variable (Blomberg et al. 2012). Typically, standard statistics for linear models are derived from PGLS and tested against *t* and *F* distributions with degrees of freedom adjusted by the number of parameters being estimated. Additional reduction of the degrees of freedom is advisable when a phylogeny contains polytomies (Purvis and Garland 1993; Garland and Díaz-Uriarte 1999) as comparative methods (including PGLS) assume a bifurcating phylogeny where descendent branches are independent. Another situation in which a reduction in the degrees of freedom might be merited is when using categorical traits as predictive variables whereby few changes of the trait character (on the phylogeny) might reduce independence in the number of comparisons among tip taxa. Strangely, there is no explicit discussion of this potential statistical issue for PGLS in the phylogenetic literature: Grafen (1989) discusses other situations in which degrees of freedom might be adjusted, Garland et al. (1993) provide a detailed worked example of a binary trait with one character change

on the phylogeny (and no adjustment to degrees of freedom), and Ives and Garland (2010, p. 10) present methods specific to binary response variables and indicate that standard PGLS approaches can be used with binary predictive variables. As an alternative to PGLS, however, Paradis and Claude (2002) propose using generalized estimating equations (GEE). GEE is a method that implements generalized linear models for correlated data. A correlation matrix derived from the phylogeny describes the marginal dependence among observations, and the method can accommodate multivariate models including both categorical and continuous variables as well as polytomies on the phylogenetic tree. In phylogenetic GEE, degrees of freedom are adjusted based on the phylogeny that uses branch lengths and also reduces for polytomies.

Here, we use both PGLS and GEE to test the effect of reproductive strategy on population genetic structure. Conventional allocations for degrees of freedom are applied along with bounded degrees of freedom (analogous to Garland and Díaz-Uriarte 1999). Because incorrect topologies can mislead phylogenetic comparative analyses (Revell 2010; Blomberg et al. 2012), we also retained OLS (described above). PGLS and GEE analyses based both on the backbone-constrained tree and the less constrained trees described previously were used to evaluate relationships among variables. Phylogenies were pruned to retain only relevant species. For PGLS, Brownian correlation matrices were derived from the phylogenies and used in generalized least squares regressions.

We also tested the prediction that the benthic guarding trait promotes diversification in two ways using family as the unit of replication. First, we tested reproductive strategy as a univariate predictor of species richness (ln transformation of valid species number) for families of reef fishes; if species richness for most clades reflects an equilibrium between speciation and extinction (Ricklefs 2007; Rabosky 2009), then species richness is a suitable response variable reflecting the balance of speciation and extinction over evolutionary time. However, if diversification is time dependent, then the relative ages of clades should be taken into consideration. Therefore, we also evaluated the effect of time independently from the effect of the dispersal trait on species richness (rather than dividing by clade age: Isaac et al. 2003).

If reproductive strategy promotes diversification (species richness), we expected it to do so indirectly by modifying population genetic structure and therefore increasing opportunities for speciation. We tested the effect of genetic structure (in combination with divergence and reproductive strategy) on species richness by averaging the predicted and residual transformed F_{ST} values based on mtDNA sequence data within families (80 species from 25 families) and using these as explanatory variables in a linear model of ln(species richness) and in a similar PGLS model which included the effect of phylogeny. We used the predicted values of F_{ST} because they included the direct effects of marker and geographic extent and we wanted to separate these effects from the residual genetic structure. This analysis was restricted to mtDNA estimates of F_{ST} to avoid introducing genetic marker as an extra variable.
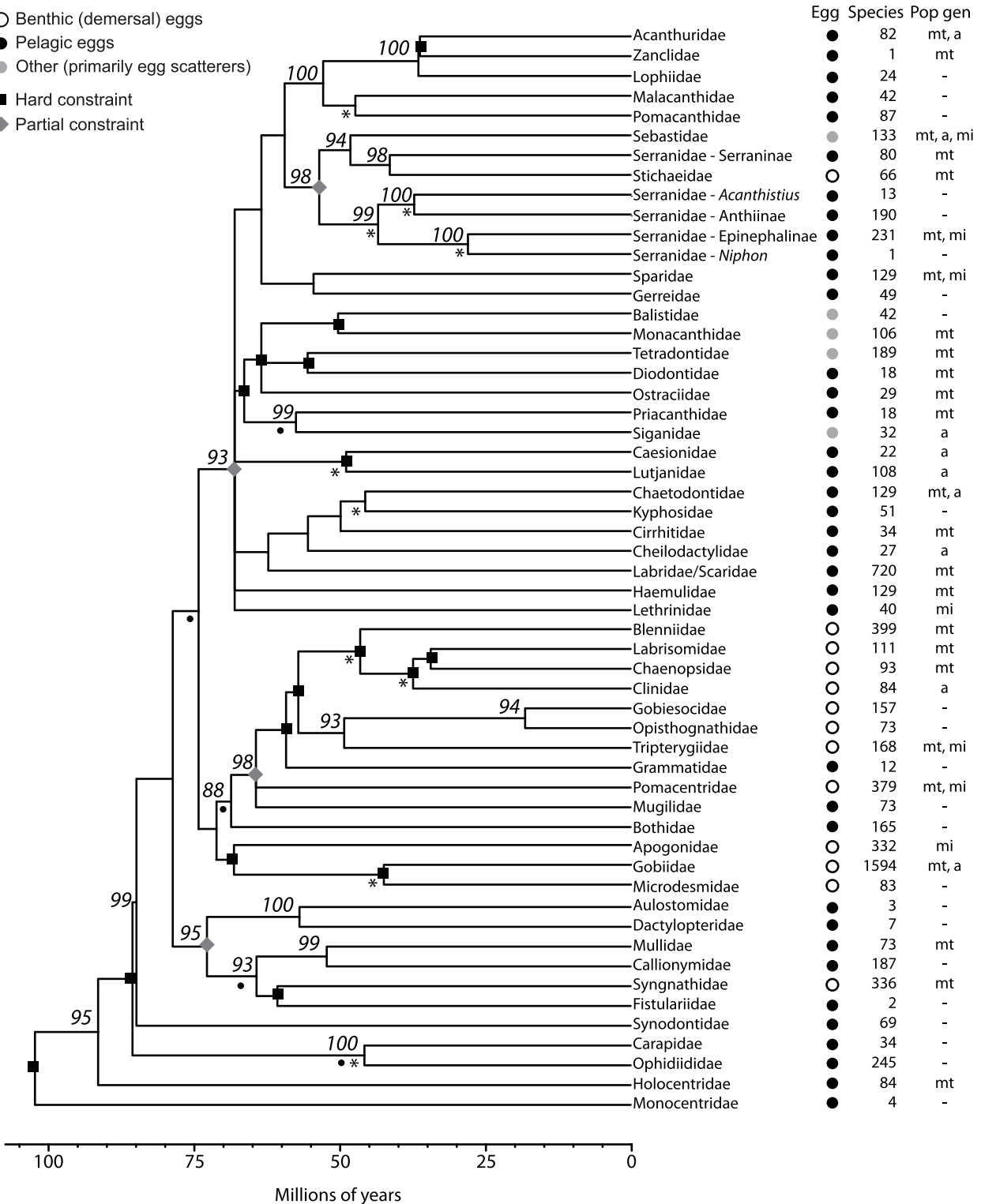
Although the benthic guarding/pelagic spawning trait is consistent within taxonomic families of reef fishes, benthic guarding is the most common reproductive mode for freshwater fishes (Helfmann et al. 2009), and freshwater fishes are absent from our trees. Thus, we are unable to estimate the phylogenetic inertia (λ, or the degree to which the phylogeny describes the variation and covariation of the data; Pagel 1999) of this trait. The λ for species richness was estimated across our focal fish families, and λ of population genetic structure was also estimated by taking the mean value of individual species' F_{ST} per family for the families that had F_{ST} estimates. All statistical analyses were conducted using R (R Development Core Team 2009), with the geiger (Harmon et al. 2009) and ape (Paradis et al. 2004) packages. Data underlying all tables and figures are deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.522cm (Riginos 2014).

Results

Phylogenetics

The highly constrained phylogenetic tree and the less constrained tree (only families and percomorphs constrained to monophyly) differed in notable aspects, especially with regard to the benthic guarding character state. Whereas in the backbone-constrained phylogenetic tree there were five clades with benthic guarding (fig. 1), in the less constrained tree there were seven clades with the benthic guarding state. Thus, the backbone-constrained tree is more conservative test of our hypotheses and probably a closer fit to the true acanthomorph evolutionary history. It is a good match to the tree from Near et al. (2013), but it also is consistent with another recent multilocus nuclear gene phylogeny proposed by Betacur et al. (2013). Although benthic guarding of eggs appears only a few times as a trait in our trees, a conclusion of limited trait changes is misleading and arises from our exclusion of freshwater and brackish water families (excluded because population genetic structure and diversification processes were expected to differ markedly from the more homogeneous marine habitats). Indeed across the acanthomorphs, benthic guarding is more common than pelagic spawning (Helfmann et al. 2009). The clades with benthic guarding in figure 1 are also present in both Near et al. (2013) and Betacur et al.'s (2013) trees and composed exclusively of marine taxa with the benthic guarding char-

Figure legend:

○ Benthic (demersal) eggs
● Pelagic eggs
● Other (primarily egg scatterers)
■ Hard constraint
◆ Partial constraint

Taxon	Egg	Species	Pop gen
Acanthuridae	●	82	mt, a
Zanclidae	●	1	mt
Lophiidae	●	24	-
Malacanthidae	●	42	-
Pomacanthidae	●	87	-
Sebastidae	●	133	mt, a, mi
Serranidae - Serraninae	●	80	mt
Stichaeidae	○	66	mt
Serranidae - *Acanthistius*	●	13	-
Serranidae - Anthiinae	●	190	-
Serranidae - Epinephalinae	●	231	mt, mi
Serranidae - *Niphon*	●	1	-
Sparidae	●	129	mt, mi
Gerreidae	●	49	-
Balistidae	●	42	-
Monacanthidae	●	106	mt
Tetradontidae	●	189	mt
Diodontidae	●	18	mt
Ostraciidae	●	29	mt
Priacanthidae	●	18	mt
Siganidae	●	32	a
Caesionidae	●	22	a
Lutjanidae	●	108	a
Chaetodontidae	●	129	mt, a
Kyphosidae	●	51	-
Cirrhitidae	●	34	mt
Cheilodactylidae	●	27	a
Labridae/Scaridae	●	720	mt
Haemulidae	●	129	mt
Lethrinidae	●	40	mi
Blenniidae	○	399	mt
Labrisomidae	○	111	mt
Chaenopsidae	○	93	mt
Clinidae	○	84	a
Gobiesocidae	○	157	-
Opisthognathidae	○	73	-
Tripterygiidae	○	168	mt, mi
Grammatidae	●	12	-
Pomacentridae	○	379	mt, mi
Mugilidae	●	73	-
Bothidae	●	165	-
Apogonidae	○	332	mi
Gobiidae	○	1594	mt, a
Microdesmidae	○	83	-
Aulostomidae	●	3	-
Dactylopteridae	●	7	-
Mullidae	●	73	mt
Callionymidae	●	187	-
Syngnathidae	○	336	mt
Fistulariidae	●	2	-
Synodontidae	●	69	-
Carapidae	●	34	-
Ophidiididae	●	245	-
Holocentridae	●	84	mt
Monocentridae	●	4	-

Millions of years (scale: 100, 75, 50, 25, 0)

Node support values: 100, 100, 94, 98, 98, 99, 100, 100, 99, 93, 95, 93, 99, 95, 88, 98, 93, 94, 100, 99, 100

acter state. Therefore, there is no evidence for trait reversals within these clades hidden by the omission of some in-group taxa, but along the deeper nodes of the phylogeny, it is likely that many ancestral nodes would have a benthic guarding state.

Reproductive Strategy as a Predictor of Population Genetic Structure and Species Richness

Consistent with the expectation that dispersal ability should affect both population genetic structure and diversification, we found that reproductive strategy (benthic guarded or pelagic spawned) of reef fishes is a strong predictor of population genetic structure and species richness. In linear models of population structure, benthic guarding species had significantly greater F_{ST} values ($P \leq .0109$) in OLS (107 species from 22 families) and GEE (91 species from 22 families) models that also included the geographic extent over which F_{ST} was estimated (table 1, fig. 2*A*), regardless of whether an interaction term between marker and geographic extent was included (table 1). For PGLS models, benthic guarding species also had substantially greater population structure (P = .0351, without the marker × geographic extent interaction term, df = 86; P = .0533 with the interaction term, df = 84, using two-tailed tests). Probabilities for PGLS models based on bounded degrees of freedom (analogous to Garland and Díaz-Uriarte 1999) were significant at the 0.05 threshold for the effect of reproductive strategy on F_{ST} for df $\geq$ 6 in one-tailed tests (threshold of df = 4 without the marker by geographic extent interaction term, threshold of df = 6 with the interaction term), and in two-tailed tests the effect of reproductive strategy was significant when df $\geq$ 6 but not significant when the interaction term was included. Similarly, for the effect of reproductive strategy on species richness, probabilities were less than 0.05 for df $\geq$ 4 in one-tailed tests and df $\geq$ 8 in two-tailed tests. Because there are five clades with the benthic guarding reproductive strategy, the most conservative choice for degrees of freedom would be df = 4, but this is certain to be an underestimate of the true degrees of freedom as benthic guarding is common among freshwater fishes, suggesting many character state changes along the full acanthomorph tree. Probability curves for the bounds of possible degrees

of freedom are shown in a supplementary figure contained in the Dryad data package, http://dx.doi.org/10.5061/dryad.522cm (Riginos et al. 2014).

Table 1 presents the GEE and PGLS models based on the backbone-constrained phylogeny (fig. 1), which matches Near et al. (2013), where five clades have the egg-guarding character trait. For our less constrained tree, where there were seven clades with the benthic egg-guarding trait, *t* values for the reproductive strategy variable had greater absolute values and lower associated *P* values ($P \leq .0034$: see supplemental table 1 in the Dryad data package, http://dx.doi.org/10.5061/dryad.522cm; Riginos et al. 2014) as compared to the constrained tree in all GEE and PGLS models. Removing either or both ambiguous trait groups (egg scatterers and Sebastids) from the models had little effect on the overall results, with reproductive strategy remaining a significant predictor of F_{ST} in models that included geographic extent as a covariate based on OLS ($P \leq .0007$), GEE ($P \leq .0188$), and PGLS ($P \leq .0435$) using either the backbone-constrained or unconstrained tree. Similarly, reproductive strategy was a significant predictor of F_{ST} in analyses restricted to mtDNA sequence based studies with OLS ($P = .0012$, $n = 80$ species), with GEE on both the constrained and unconstrained trees ($P \leq .0222$, $n = 67$) and with PLGS on the unconstrained tree ($P = .0093$, $n = 67$ species) but not the backbone-constrained tree ($P = .0776$, $n = 67$ species; for the model ln[Rousset]~marker + ln[km] + egg).

Reproductive strategy also significantly predicted species richness of fish families (table 2;, fig. 2*B*), with benthic guarding families being more species rich than pelagic spawning families. Relative family clade depth, as estimated by maximum *COI* divergence within a family, was also a highly significant predictor of species richness (table 2), implying that older clades are more species rich than younger clades. Clade depth alone, however, was a weaker predictor of species richness when compared to models that included both clade depth and reproductive strategy, as assessed by Akaike Information Criterion values for OLS and PGLS, with little difference between models in GEE (table 2). Excluding egg scatterers and Sebastids and/or families with *COI* divergence less than 0.2% (including the monotypic families Zanclidae and *Niphon spinosus*, which was treated as a monotypic family following Smith

q4

Figure 1: Chronogram of the 55 focal families. This is a reduced version of our full Bayesian tree to illustrate relationships among taxonomic families. Constraints were implemented to be consistent with the phylogeny of Near et al. (2013), where hard constraints enforced monophyly of all descendant taxa from the indicated node and partial constraints enforced monophyly of some descendant taxa from that node but allowed other (unassigned) taxa to be added to the clade. Branches with posterior probabilities greater than 80% are indicated. Dots below nodes indicate that the same node was present but not well supported in the Near et al. (2013) phylogeny, and asterisks indicate that the same node was also recovered in our less constrained tree search. Reproductive strategy, valid species numbers, and available population genetic data (mt = mitochondrial DNA sequences, a = allozymes, and mi = microsatellites) are shown per family.

Table 1: Reproductive strategy and geographic extent predict population genetic structure in reef fish species

Model, terms	Ordinary linear models n = 107				Generalized estimating equations[a] n = 91, phylogenetic df = 39.8				Phylogenetic generalized least squares[a] n = 91			
	Effect size	t	P^b	AIC	Effect size	t	P^b	QIC	Effect size	t	P^b	AIC
$\ln(\text{Rousset } F_{ST}) \sim \text{marker} + \text{egg}$:												
Intercept (marker—allozyme, egg-benthic)	−2.65	−4.47	<.0001	467.1	−1.08	−1.07	.2924	396.8	−1.08	−.37	.7133	501.2
Marker—microsatellite	−.48	−.59	.5553		−1.53	−5.42	<.0001		−1.53	−1.87	.0648	
Marker—mtDNA sequence	1.47	2.50	.0141		.47	1.37	.1777		.47	.47	.6364	
Reproduction—pelagic spawning	−.82	−2.03	.0457		−1.30	−1.72	.0948		−1.30	−.59	.5554	
$\ln(\text{Rousset } F_{ST}) \sim \text{marker} + \ln(\text{km}) + \text{egg}$:												
Intercept (marker—allozyme, egg-benthic)	−8.48	−5.81	<.0001	451.2	−16.36	−11.7	<.0001	514.4	−16.36	−8.05	<.0001	407.5
Marker—microsatellite	−.09	−.11	.9096		−.70	−2.16	.0378		−.70	−1.48	.1433	
Marker—mtDNA sequence	.57	.99	.3255		−.37	−.94	.3527		−.37	.64	.5211	
$\ln(\text{km})$	.88	4.31	<.0001		2.03	19.51	<.0001		2.03	13.35	<.0001	
Reproduction—pelagic spawning	−1.62	−3.86	.0002		−2.70	−3.13	.0035		−2.70	−2.14	.0351	
$\ln(\text{Rousset } F_{ST}) \sim \text{marker} + \ln(\text{km}) + \text{egg} + \text{marker:} \ln(\text{km})$:												
Intercept (marker—allozyme, egg-benthic)	−5.45	−1.22	.2247	447.7	−6.13	−1.37	.1793	445.6	−6.13	−1.00	.3221	386.0
Marker—microsatellite	2.13	.42	.6771		2.88	.58	.5652		2.88	.42	.6743	
Marker—mtDNA sequence	−5.16	−1.09	.2804		−13.07	−2.98	.0054		−13.07	−2.17	.0332	
$\ln(\text{km})$	.46	.74	.4623		.55	.94	.3555		.55	.68	.4982	
Reproduction—pelagic spawning	−1.66	−4.04	.0001		−2.19	−2.70	.0109		−2.19	−1.96	.0533	
Marker—microsatellite: $\ln(\text{km})$	−.36	−.50	.6168		−.58	−.87	.3888		−0.57	−.63	.5278	
Marker—mtDNA seq: $\ln(\text{km})$	.76	1.16	.2483		1.71	2.89	.0069		1.71	2.09	.0392	

Note: AIC = Akaike Information Criterion, QIC = quasi-likelihood information criterion.

[a] Phylogeny is backbone-constrained tree. See text for details.
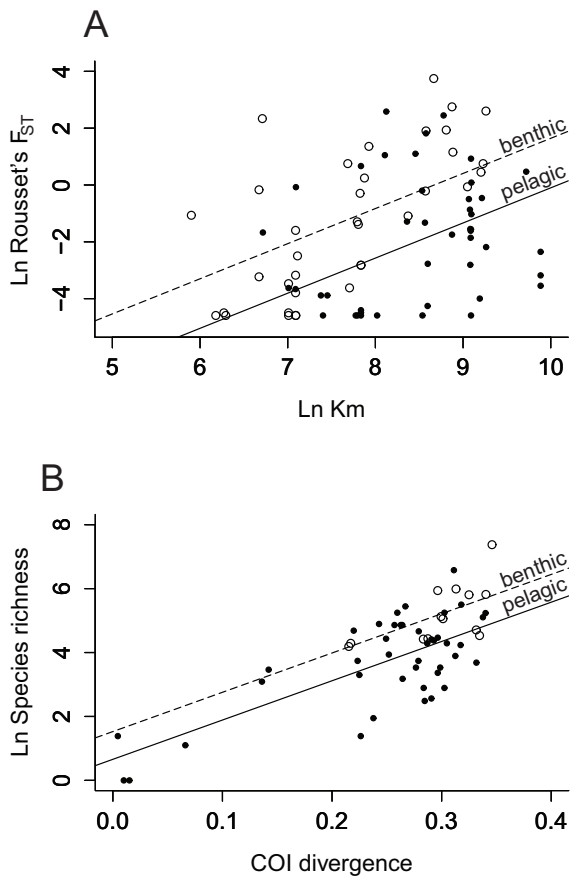
[b] Based on two-tailed tests.

Discussion

Despite the central role of gene flow in evolution, there are few dispersal traits demonstrated to have a statistically significant effect on both micro- and macroevolutionary patterns of diversity. Here we show that the major reproductive strategies of reef fishes (i.e., benthic guarding vs. pelagic spawning) significantly contribute to both intraspecific genetic differentiation and species richness in families. Consistent with benthic guarders dispersing less than pelagic spawners, we find greater genetic differentiation over geographic distance for benthic guarders (fig. 2*A*). We also show that benthic guarding families are more speciose than families of pelagic spawners but that clade depth is an additional predictor of species richness (fig. 2*B*).

Although there have been many attempts to link dispersal traits to intraspecific genetic differentiation, the results have been variable and often contradictory (Loveless and Hamrick 1984; Hamrick and Godt 1996; Bilton et al. 2001; Duminil et al. 2007; Bradbury et al. 2008; Burney and Brumfield 2009; Kelly and Palumbi 2010; Riginos et al. 2011; Selkoe and Toonen 2011; Dawson et al., forthcoming). Moreover, many studies have ignored or only partially corrected for phylogenetic relationships, for example, restricting analyses to higher order taxonomic groups (noteworthy exceptions include Duminil et al. 2007; Burney and Brumfield 2009). Thus, our study is significant for establishing an association between a specific trait and population genetic differentiation that appears robust to phylogenetic relationships and uncertainty regarding those relationships. We also predicted and demonstrated an inverse relationship between presumed dispersal ability and species richness, a finding mirrored in some studies of birds (Belliure et al. 2000; Claramunt et al. 2012) and plants (Tiffney and Mazer 1995; Bolmgren and Eriksson 2005) but not others (Owens et al. 1999; Price and Wagner 2004; Phillimore et al. 2006).

Considering the relationship between dispersal, genetic differentiation, and species diversity in combination, our results match the expectation that macroevolutionary patterns of diversity should arise from within-species dynamics, with key traits as potential ultimate causes (Heard and Hauser 1995; Coyne and Orr 2004; Jablonski 2008). For reef fishes, the effect of reproductive strategy on familial species richness significantly contributes to species richness and outweighs any intermediate association between population genetic structure and species richness. Thus, our results contrast with those from Cretaceous gastropods, where the feeding state of planktonic larvae did not contribute an additional statistical effect to species duration when range size was jointly considered with feeding state (Jablonski and Hunt 2006). These contrasting results from fishes and gastropods highlight multiple steps of cause and effect, whereby

Figure 2: Reproductive strategy, population genetic structure, and species richness. *A*, Population genetic structure of species by geographic sampling extent for studies based on mitochondrial sequences. Both the slope of the regression ($m = 1.2$, $t = 4.6$, $P < .0001$) and the difference in intercept (benthic egg-pelagic $= 1.7$, $t = 3.4$, $P = .0123$) are significant ($n = 80$). *B*, Species richness of families by maximum cytochrome oxidase I divergence within each family. Both the slope of the regression ($m = 12.39$, $t = 7.3$, $P < .0001$) and the difference in intercept (benthic egg-pelagic $= 0.9$, $t = 2.7$, $P = .0098$) are significant ($n = 55$). Large open circles indicate benthic guarded eggs (dashed regression lines), and small solid circles indicate pelagic spawned eggs (solid regression lines).

and Craig 2007) in any combination did not affect overall results, with both reproductive strategy and clade depth remaining significant predictors of species richness ($P \leq .022$ for reproductive strategy and $P \leq .0095$ for clade depth across OLS, GEE, and PGLS models with either phylogenetic tree). The F_{ST} averaged within families did not affect species richness, directly indicating that reproductive strategy is a sufficient predictor of species richness. The phylogenetic signal of species-level population structure was weak and not significantly different from zero, and similarly species richness was not discernibly correlated with the phylogeny ($\lambda < 0.001$, NS).

Table 2: Reproductive strategy and clade depth predicts species richness of families

Model	Terms	Ordinary linear models (n = 55)				Generalized estimating equations[a] (n = 91, phylogenetic df = 31.4)				Phylogenetic generalized least squares[a] (n = 55)			
		Effect size	t	P[b]	AIC	Effect size	t	P[b]	QIC	Effect size	t	P[b]	AIC
ln(spp_richness)~ Egg	Intercept (egg-benthic)	5.21	13.83	<.0001	193.6	4.96	6.79	<.0001	126.2	4.95	4.89	<.0001	207.6
	Reproduction: pelagic spawning	−1.47	−3.42	.0012		−1.50	−3.22	.0031		−1.50	−2.32	.0244	
ln(spp_richness)~ Clade_depth	Intercept(clade_depth = 0)	.53	1.13	.265	161.8	.49	.90	.0375	78.8	.49	.69	.4947	166.8
	Clade_depth	13.61	7.90	<.0001		14.2	10.70	<.0001		14.24	8.18	<.0001	
ln(spp_richness)~ Clade_depth + egg:	Intercept(clade_depth = 0, egg-benthic)	1.50	2.61	.0117	156.7	1.55	2.50	.0184	79.6	1.56	1.97	.0540	162.2
	Clade_depth	12.39	7.31	<.0001		13.78	10.51	<.0001		13.78	8.28	<.0001	
	Reproduction: pelagic spawning	−.85	−2.68	.0098		−1.11	−3.28	.0027		−1.11	−2.59	.0125	

Note: AIC = Akaike Information Criterion, QIC = quasi-likelihood information criterion.

[a] Phylogeny is backbone-constrained tree. See text for more details.

[b] Based on two-tailed tests.

correlations between an ultimate effect (macroevolutionary diversification) and potential ultimate causes (key traits) may be detectable in some instances (reef fishes) but mitigated or outweighed in other instances.

The complex and seemingly contradictory results across study systems regarding relationships between dispersal, population genetic structure, and species richness may arise from three related factors. First, the influence of a dispersal trait is likely to depend upon the ecological context. For example, plants with fleshy seeds exhibit greater species richness in forest taxa but not for taxa dominated by herbs (Tiffney and Mazer 1995; Smith 2001; Bolmgren and Eriksson 2005); similarly, the effects of dispersal barriers differs by habitat usage for tropical birds (greater genetic differentiation across barriers for understory as compared to canopy birds; Burney and Brumfield 2009) and by dispersal trait for reef fishes (greater differentiation across barriers for benthic guarded species; Riginos et al. 2011). Thus, the multivariate effects on emergent response variables, such as population genetic structure and diversification, can obscure their relationship with dispersal traits. Second, genetic measures are notoriously poor for distinguishing among low levels of gene flow (Waples 1998; Whitlock and McCauley 1999), and yet the parameter space between low and no gene flow (or dispersal) is important for speciation (Coyne and Orr 2004) and perhaps the balance of speciation and extinction (Gaston and Chown 1999; Paulay and Meyer 2006; Claramunt et al. 2012). Finally, our human intuitions regarding dispersal traits might be quite poor for rare gene exchange events (including long-distance dispersal) and important traits for rare gene exchange may differ from ordinary dispersal

dynamics amenable to direct measurements (e.g., in the near-to-mid range of a dispersal kernel).

Several features of our study system presumably underpin the significant and consistently detectable effect of the benthic guarding/pelagic spawning trait on both population genetic structure and species richness for reef fish families. First, our trait categories are aggregate descriptors for a suite of correlated reproductive aspects that may act in concert to affect dispersal. Guarded benthic eggs tend to be larger and hatch larger, stronger swimming larvae able to maintain proximity to shoreline as compared to larvae from pelagic eggs (reviewed by Leis 1991; Cowen and Sponaugle 1997). Additionally, the pelagic larval duration of benthic egg species is probably shorter relative to pelagic egg species (Cowen and Sponaugle 1997). Thus, it is expected that the average distance traveled by larvae from benthic eggs is less than the distance traveled by larvae from pelagic eggs. Furthermore, the greater reproductive output of pelagic spawners should increase the number of dispersing larvae and therefore increase the total number of long-distance dispersal events for those species (Treml et al. 2012). Any and all of these life-history aspects may independently affect dispersal frequencies. Secondly, the majority of dispersal for reef fishes occurs during the brief period from egg to juvenile settlement (analogous to seed dispersal in plants) with relatively little movement of adults, so early life history categories effectively describe lifetime movement. For terrestrial animals, especially mammals and birds, dispersal is greatly affected by social interactions (Greenwood 1980; Clutton-Brock and Lukas 2012) and thus not necessarily easy to categorize based on discrete traits. Third, we improved our linear

models of population genetic structure by including the geographic sampling extents of the original studies as a covariate, which greatly increased the model fit to the data over models that do not include geographic extent (table 1). This is not a unique attribute of our study but may aid in capturing some of the spatial processes contributing to genetic differentiation. Finally, although our replication of population genetic structure within families is low (fig. 1) we have good replication at the level of family (population genetic data for 32 families, species richness for 55 families) and it is at the broad level of phylogeny and taxonomy that we find an effect of reproductive strategy. In contrast, Kisel et al. (2012) found no correlation between population genetic structure and species richness comparing across five orchid clades. Thus, it may be more fruitful to increase replication across evolutionary space rather than increasing precision within lineages and taxonomic groups.

An unexpected result from our analyses was that family clade depth, estimated by maximum *COI* genetic distance within each clade, was also a significant predictor of species richness. This positive correlation is expected if diversification (i.e., the net difference between speciation and extinction rates) remains constant or increases through time within lineages. However, recent theoretical treatments question the validity of this expectation, and reanalyses of phylogenetic data (including for teleost fishes; Rabosky 2009) show that for most taxa there is no relationship between species richness per clade and clade age. An alternative explanation is that rates of molecular evolution are correlated with diversification (Webster et al. 2003), as has been observed for reptiles and birds (Eo and DeWoody 2010; Lanfear et al. 2010) and plants (Barraclough and Savolainen 2001; Duchene and Bromham 2013). Because there is no reason to suspect that the *COI* locus is directly involved in speciation, the association between species richness and maximal branch length within fish clades could reflect a common cause; for instance, the process of speciation itself might affect substitution rates, with reductions in population sizes being one plausible explanation for increased rates of molecular evolution (Barraclough and Savolainen 2001; Venditti and Pagel 2010). If this were the case, we might expect greater clade depth among benthic egg fishes associated with a greater propensity for speciation, however a post hoc test for a difference in *COI* divergence found that divergence among benthic guarders was not significantly greater than for pelagic spawners ($t = 0.78$, $P = .4$, df $= 53$, with PGLS based on the constrained tree). Therefore, it seems most plausible that substitution rates have been fairly constant within lineages.

Reductions in gene flow are integral to the predominant models of speciation (Coyne and Orr 2004). Therefore factors reducing gene flow should increase genetic differentiation within species and thereby result in greater rates of speciation. Here, we demonstrate that a key life-history trait affects both population genetic structure and species richness. Hence, our results uphold the simple and long-standing prediction that macroevolutionary diversification is shaped by emergent properties of species arising from their key traits. For reef fishes, this detectable evolutionary connection between a dispersal trait, population genetic structure, and species richness implies that reductions in gene flow associated with dispersal traits and geographic isolation facilitate speciation. More generally, our results suggest that the relevant geographic distance for gene flow and speciation scales with an organism's dispersal capacity and can sometimes be qualitatively predicted from species' life history. Thus, dispersal-related traits affect the evolutionary trajectory of species both in the short and long term.

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