

Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in *Drosophila* and birds

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The rate at which speciation occurs varies greatly among different kinds of organisms and is frequently assumed to result from species- or clade-specific factors that influence the rate at which populations acquire reproductive isolation. This premise leads to a fundamental prediction that has never been tested: Organisms that quickly evolve prezygotic or postzygotic reproductive isolation should have faster rates of speciation than organisms that slowly acquire reproductive isolation. We combined phylogenetic estimates of speciation rates from *Drosophila* and birds with a method for analyzing interspecific hybridization data to test whether the rate at which individual lineages evolve reproductive isolation predicts their macroevolutionary rate of species formation. We find that some lineages evolve reproductive isolation much more quickly than others, but this variation is decoupled from rates of speciation as measured on phylogenetic trees. For the clades examined here, reproductive isolation—especially intrinsic, postzygotic isolation—does not seem to be the rate-limiting control on macroevolutionary diversification dynamics. These results suggest that factors associated with intrinsic reproductive isolation may have less to do with the tremendous variation in species diversity across the evolutionary tree of life than is generally assumed.

mechanism of speciation | hybrid incompatibility | speciation gene | species concept

A central challenge at the interface between macroevolution and microevolution is to explain the population-level processes that contribute to biological variation in diversification rates and species richness (1). Phylogenetic evidence for biological variation in the rate of species diversification is widespread (2, 3), and numerous studies have now linked specific traits to the dynamics of speciation and extinction as realized over macroevolutionary timescales (2, 4). At the population level, a microevolutionary research program on the biology of speciation has focused on the factors that lead to various forms of reproductive isolation (RI) between populations (5, 6). Explaining how and why RI evolves is generally considered to be the central and defining challenge in the study of speciation (2, 7–9), and recent studies have made great progress toward explaining the genetic and ecological basis for various forms of RI (7, 8, 10).

Most microevolutionary research on speciation implicitly assumes that RI is the defining and rate-limiting step in the speciation process (7, 8, 11), but the evolution of RI need not bear any predictive relationship to rates of species diversification as realized over macroevolutionary timescales (12). This has long been recognized by the paleontological community, where “successful” speciation is believed to entail not only the evolution of reproductive isolation but also the persistence of incipient species (13–15). For example, speciation might be limited primarily by the rate at which lineages form allopatric isolates (6, 16) or by the capacity for geographic range expansion (17, 18). Likewise, speciation might be limited more by factors that influence the temporal persistence of reproductively isolated populations

(15, 19) than by the rate at which RI itself evolves. Finally, macroevolutionary diversity dynamics might be regulated primarily by factors that influence extinction rates (4). These factors need not be independent of reproductive isolation. For example, reproductive barriers can reduce the probability of population fusion following secondary contact between nascent species, thus exerting a direct effect on population persistence (2). Likewise, RI can influence the dynamics of geographic range evolution, which may in turn have secondary consequences for rates of demographic extinction and allospecies formation (17, 18).

Here, we provide a direct test of the relationship between reproductive isolation and macroevolutionary diversification. If the widespread variation observed in macroevolutionary diversification rates (20, 21) is attributable to factors that cause RI (2, 22, 23), then it must also be true that lineage-specific differences in the rate at which RI evolves will influence large-scale patterns of species diversification. In this context, a species with a “fast” rate of RI evolution will, all else being equal, form more reproductively isolated lineages than a species with a “slow” rate of RI evolution.

We use data from the two groups of animals for which the most extensive multispecies RI data have been compiled and for which we could derive phylogeny-based estimates of species diversification rates. The first dataset is an update of Coyne and Orr’s (2) seminal work on the relationship between genetic distance, geographic status, and reproductive isolation in *Drosophila* (24) and contains estimates of both prezygotic and intrinsic postzygotic RI. The second dataset includes measurements of intrinsic postzygotic isolation from interspecies hybridizations in birds (25, 26). We developed a modeling framework for estimating species- and clade-specific differences in the rate of evolution of RI and applied it to both birds and flies to test

Significance

Rates of species diversification vary widely in the natural world, leading to profound differences in species richness among different kinds of organisms. Variation in the rate at which new species arise is frequently assumed to result from factors that influence the rate at which populations become reproductively isolated from each other. We tested this assumption in *Drosophila* flies and birds. Surprisingly, we find no evidence that the propensity of organisms to evolve reproductive isolation predicts the rate at which they form new species over geological timescales. These results suggest that factors that cause intrinsic reproductive isolation may play less of a role in explaining biological diversity than generally assumed.

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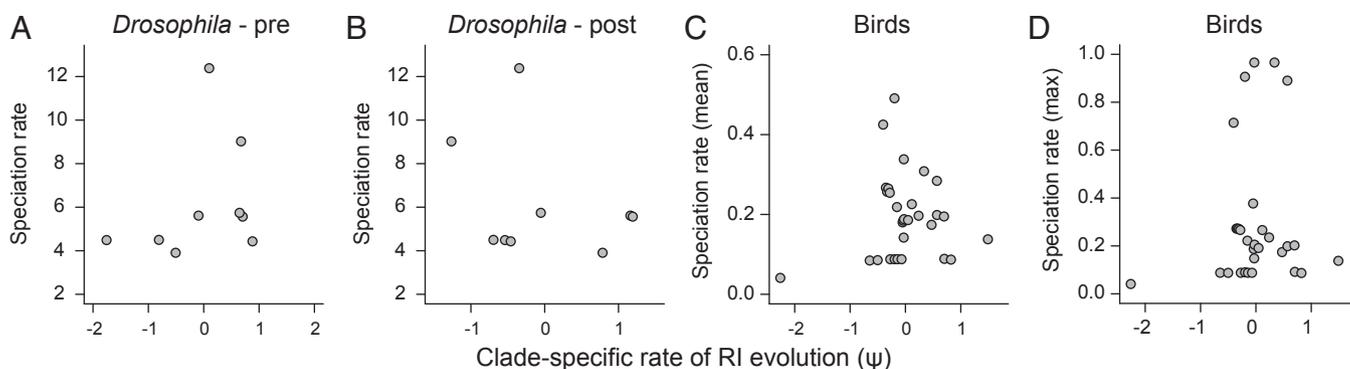


Fig. 3. Rates of speciation are uncorrelated with clade-specific variation in the rate at which species accumulate RI (Table 1; Table S3). (A) Premating ψ and relative speciation rate across nine *Drosophila* clades assuming low relative extinction rates (ψ model: asymptotic). (B) Postzygotic ψ for *Drosophila* (linear model) and relative speciation rates. (C) Postzygotic ψ estimates and mean speciation rate across 30 avian families (ψ model: asymptotic). (D) Postzygotic ψ estimates and the maximum speciation rate for bird clades.

of species origination and extinction as realized over geological time to specific mechanisms that occur within and between populations at ecological timescales. Previous studies have speculated that some biological traits might influence macroevolutionary dynamics via their effects on the rates at which populations evolve reproductive isolation (2, 22, 23, 36). By quantifying lineage-specific variation in the rate at which species accumulate RI, our study provides a direct test of the relationship between RI and macroevolutionary diversification. If the causes of speciation at macroevolutionary scales are merely the long-term manifestation of processes we typically study at microevolutionary scales, we should observe a coupling between rates of evolution of RI and macroevolutionary diversification. We found that clades of both flies and birds differ significantly in the rate at which they evolved intrinsic postzygotic isolation, but this variation is decoupled from macroevolutionary diversification dynamics. For *Drosophila*, we also found a lack of relationship between pre-mating sexual isolation and macroevolutionary diversification, although the differences in rates of pre-mating RI evolution across *Drosophila* clades are not significant.

Our results bear most directly on the relationship between intrinsic genetic incompatibilities and speciation. The genetic factors that contribute to hybrid inviability and sterility have been widely studied, owing in large part to the relative ease with which controlled laboratory crosses can be performed between different species (2, 37, 38). Most researchers recognize that RI can take many forms, but genetic incompatibilities are nonetheless

assumed to play an important role in speciation (2, 39, 40). Genetic incompatibilities have even been proposed to play a role in bird speciation (37, 41), where sexual and ecological isolation is common between closely related species pairs (37). Interestingly, the idea that postzygotic isolation contributes little to the onset of bird speciation was suggested previously (42) but had not been tested. Our results constitute a formal test of this idea and suggest that hybrid incompatibilities in birds may have little to do with the macroevolutionary realization of the speciation process.

Given the observed levels of variation in macroevolutionary diversification rates and rates of postzygotic RI evolution across clades, we find it surprising that we see no hint of a relationship between these quantities (Table 1 and Fig. 3 and Table S3). Reproductive isolation data are notoriously noisy (e.g., Fig. 1), but our power simulations (Fig. S6) indicate that moderate correlations between postzygotic ψ and the rate of speciation could have been inferred under our analytical protocol. It is true that weaker correlations between RI and speciation would have been more difficult to detect in these datasets, and future work may yet find that the rate of evolution of RI plays at least a minor role in shaping variation in speciation rates in these clades. We found little evidence for clade-specific variation in pre-mating ψ for *Drosophila*, suggesting that the causes of variation in macroevolutionary diversification among *Drosophila* clades is unlikely to be explained by differences in the rate at which clades evolve pre-mating isolation. The overall structure of pre-mating

Table 1. Relationship between macroevolutionary diversification rate (λ) and the rate of prezygotic and postzygotic evolution (ψ) for major clades of flies and birds

Group	Model	Metric	Pearson correlation (P value)	Spearman correlation (P value)	PGLS slope	PGLS P value	df
<i>Drosophila</i> (pre)	Asymptotic	λ_0	0.3 (0.43)	0.23 (0.55)	1.03	0.45	9(7)
<i>Drosophila</i> (pre)	Asymptotic	λ_{95}	0.24 (0.53)	0.58 (0.11)	0.86	0.38	9(7)
<i>Drosophila</i> (post)	Linear	λ_0	-0.31 (0.42)	-0.08 (0.84)	-1.22	0.21	9(7)
<i>Drosophila</i> (post)	Linear	λ_{95}	-0.31 (0.41)	-0.10 (0.81)	-0.71	0.33	9(7)
Birds	Linear	λ	-0.01 (0.95)	0.0 (0.98)	-0.01	0.81	30(28)
Birds	Linear	λ_{MAX}	-0.03 (0.8)	0.05 (0.77)	-0.03	0.81	30(28)
Birds	Linear	λ_{q80}	-0.03 (0.87)	0.04 (0.82)	-0.01	0.77	30(28)
Birds	Asymptotic	λ	0.04 (0.83)	0.05 (0.79)	-0.01	0.67	30(28)
Birds	Asymptotic	λ_{MAX}	-0.01 (0.96)	0.12 (0.54)	-0.04	0.7	30(28)
Birds	Asymptotic	λ_{q80}	-0.01 (0.98)	0.08 (0.66)	-0.01	0.73	30(28)

Results are shown only for the best-fit functional models for the accumulation of RI with genetic distance (Table S1 and Fig. S1). Linear and asymptotic models were approximately equivalent for birds and results for both models are shown. For *Drosophila*, λ_0 and λ_{95} are constant-rate estimators of the speciation rate assuming no extinction (λ_0) or high extinction (λ_{95}). For birds, λ is the mean speciation rate within each family, λ_{MAX} is the maximum observed rate within each family, and λ_{q80} is the estimated 0.80 quantile of the distribution of rates for each family. Avian results are shown only for the Hackett backbone topology; results for Ericson topology and for all other models are given in Table S3. The BAMM model used for the avian dataset estimates speciation rates separately from extinction rates.

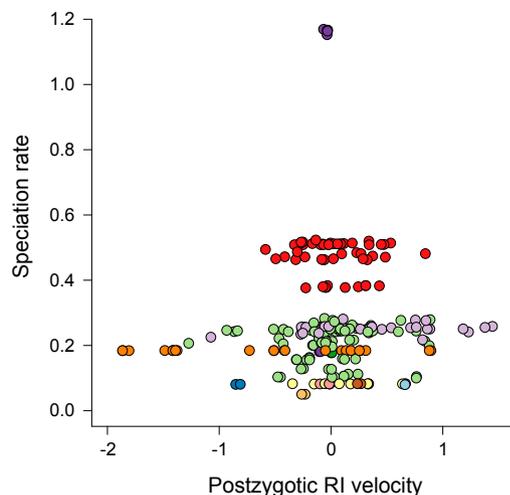


Fig. 4. Relationship between rates of speciation and species-specific velocities of postzygotic RI evolution across 244 species of birds. Instantaneous rates of speciation were estimated for each lineage under a statistical model that allowed each species to have a potentially unique rate of speciation (Fig. S5). Identically colored points denote species from the same order. Species that quickly evolve intrinsic reproductive isolation from other species do not speciate at faster rates than other species.

and postzygotic datasets for *Drosophila* is similar, and the number of crosses in the pre mating dataset exceeds that of the postzygotic dataset; we thus attribute our failure to find clade-specific differences in pre mating ψ to a lack of biologically meaningful variation in this parameter rather than lack of statistical power.

Under a strict biological species concept, speciation is defined as the evolution of reproductive isolation, but a more inclusive view of the speciation process allows for the possibility that other factors limit the rate at which speciation occurs. Many researchers have suggested that speciation might be limited primarily by factors associated with the persistence of incipient species (13–15). Models of ephemeral speciation propose that it is relatively easy for lineages to form incipient species, but the vast majority of new species do not persist over macroevolutionary timescales (15, 19). This view is closely related to Darwin's perspective on speciation, whereby ecological character divergence plays a critical role in mediating the temporal persistence of new species (43–45). Anecdotally, at least, it seems clear that many examples of rapid speciation in nature are limited by population persistence. Stickleback fishes, for example, can evolve reproductive isolation very quickly in postglacial lakes, but nascent species pairs typically fail to persist over geological timescales (46). We speculate that primary controls on species persistence may involve factors associated with geographic range expansions (17), including ecological divergence between incipient species (47) and antagonistic interactions with pathogens (48). Addressing these issues will require careful consideration of the nature of species themselves (16), and it is possible that the manner in which species are defined could influence the results of analyses such as those presented here (49).

For many decades, researchers have sought to explain the mechanisms that lead to reproductive isolation between incipient species (5, 6). Reproductive isolation clearly plays an important role in maintaining species boundaries, but there is yet no evidence that any forms of reproductive isolation influence speciation rates as realized over macroevolutionary timescales. We have described a general framework that can be used to test whether any components of reproductive isolation are associated with speciation as measured at macroevolutionary scales, provided that broadly comparative data on pairwise RI can be obtained.

Our results are based on two of the most exhaustive compilations of RI ever assembled for any group of organisms (24–26,

50) and include many decades of work by dozens of researchers. The results reported here pertain strictly to *Drosophila* and birds and suggest an acute need for empirical studies on the evolution of reproductive isolation from groups of organisms that vary widely in their rate of macroevolutionary diversification. Numerous studies have assessed components of RI at relatively fine taxonomic scales (51, 52), but we suggest that future research should explicitly target groups that are known to differ in diversification rate. At present, we do not understand the evolutionary and ecological mechanisms that underlie differential rates of species diversification in *Drosophila*, birds, and other taxa. However, a complete explanation for speciation in nature may require that we broaden our explanatory paradigm beyond the mechanisms that underlie reproductive isolation.

Materials and Methods

RI Data. We compiled literature data on RI for birds (26) and *Drosophila* (24, 50). Prezygotic (pre mating) RI in the dataset was based on laboratory copulation experiments (24), and the overall level of pre mating RI was computed as $1 - (\text{frequency of heterospecific matings})/(\text{frequency of homospecific matings})$. We used Yukilevich's (24) estimates for postzygotic RI in our analyses, which assign each species cross a value between 0 and 1, based on the percentage of sterile or inviable F_1 offspring for each interspecific cross. The full avian dataset consisted of postzygotic RI estimates from 407 hybridizations (26). The data were scored on a scale of 1–5, with 1 indicating fertility of the crossed pair, 3 indicating that F_1 s in both directions were viable but infertile, and 5 indicating that no F_1 s were viable. We converted these to a [0, 1] scale for comparison with *Drosophila*. Excluding intraspecific crosses and crosses involving species that were not present in the Jetz et al. (20) phylogeny left a total of 244 species and 287 crosses.

Phylogenies. We inferred a phylogeny for major *Drosophila* lineages using DNA sequence alignments from a recent phylogenetic study of the genus (53). We trimmed the taxon set to include 94 species that we believed to be important for estimating the relative crown-clade age for each of nine major subclades for which we could obtain estimates of species richness (*SI Materials and Methods*). We analyzed the resulting dataset of 94 taxa using an uncorrelated lognormal relaxed-clock model of sequence evolution in the program BEAST. We used two phylogenies as a framework for estimating speciation rates in birds (Fig. S5) and for avian PGLS analyses (Fig. S4). Both are derived from the Jetz et al. (20) time-calibrated phylogeny for all birds. We analyzed maximum clade credibility (MCC) trees for both backbone trees used by Jetz et al. (20). These trees contained only the 6,670 species for which genetic data were available; we did not include those species whose phylogenetic positions were estimated from taxonomic information alone (20).

Rate of RI Evolution. We assumed that each species was characterized by a specific tempo of RI evolution, which we denote by ψ . Individual ψ values interact additively, such that the expected rate of evolution of reproductive isolation from a given cross between the i 'th and j 'th species is proportional to $\psi_i + \psi_j$. In addition to the linear model described in the main text, we considered an asymptotic model, such that $Y_{ij} = \varepsilon + (\beta_0 + \delta X_{ij})/(1 + \delta X_{ij})$, where $\delta = (\beta_1 + \psi_i + \psi_j)$ and ε is sampled from the error distribution for pairwise RI observations. We also considered a quadratic model, where reproductive isolation was modeled as $Y_{ij} = \varepsilon + X_{ij}\beta_0 + (\beta_1 + \psi_i + \psi_j)X_{ij}^2$. For *Drosophila*, our model also incorporated the geographic status of species pairs (sympatric versus allopatric), because geographic status is known to influence the relationship between reproductive isolation and genetic distance (50, 54). Models to account for sympatric and allopatric taxa were identical to those described above but contained separate β_0 and β_1 parameters for sympatric and allopatric species pairs (*SI Materials and Methods*). Thus, the full linear model for the *Drosophila* datasets is given by

$$Y_{ij} = c\beta_{0,S} + (1 - c)\beta_{0,A} + (c\beta_{1,S} + (1 - c)\beta_{1,A} + \psi_i + \psi_j)X_{ij} + \varepsilon,$$

where $\beta_{0,A}$ and $\beta_{0,S}$ denote parameters for allopatric and sympatric species pairs and c is an indicator variable that takes a value of 1 if the species pair is sympatric and 0 if allopatric. We implemented all models in a Bayesian framework to estimate marginal posterior distributions and maximum a posteriori (MAP) estimates for ψ and all other parameters. We compared models using a posterior-simulation based version of the AIC criterion, known as AIC_M . AIC_M can be computed directly from MCMC simulation output as $AIC_M = 2L - 2S$, where L and S denote the mean and variance of

the posterior log-likelihoods (30). Full details of the modeling approach are given in *SI Materials and Methods*.

Diversification Rates. Using BEAST-derived estimates of crown-clade age for *Drosophila* clades, we computed estimators of net speciation rates (55) under relative extinction rates of 0 and 0.95. For PGLS analyses involving *Drosophila*, we pruned the MCC tree to include only representatives from each of the nine major clades outlined in *SI Materials and Methods*. For birds, we estimated branch-specific rates of speciation using a new Bayesian method (BAMM) for the analysis of speciation and extinction rates on molecular phylogenies (21). BAMM enables reconstruction of marginal posterior distributions of speciation and extinction rates on each branch of a reconstructed phylogenetic tree (21). The avian trees contained only 67% of avian species, and we analytically accounted for incomplete taxon sampling directly in the BAMM model itself (21). We modified BAMM to allow rates of speciation to change exponentially through time within particular shift regimes, thus allowing the model to explicitly account for diversification rate variation through time and among lineages (*SI Materials and Methods*). We performed BAMM analyses on both Hackett and Ericson backbone topologies and report all rates and correlations in *Table S3*.

Power Analyses. For each RI dataset, we simulated pseudodatasets with a known correlation between RI and macroevolutionary diversification. We first sampled ψ values for each clade from a distribution with variance identical to the observed (estimated) distribution. We then generated RI observations using the MAP parameter estimates for each dataset, including random noise sampled from the fitted error distribution. All datasets simulated in this fashion contain the same number of pairwise crosses per clade as the observed data. We then sampled diversification rates from a lognormal distribution parameterized to have the same mean and variance as the observed distribution such that the Pearson correlation between clade-specific ψ values and diversification was between 0.25 and 1.0. We estimated MAP parameter values for each simulated dataset and evaluated the correlation between the estimated ψ values and speciation rates. A total of 2,000 simulations were performed for each dataset (*SI Materials and Methods* and *Fig. S6*).

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- Kisel Y, et al. (2012) Testing the link between population genetic differentiation and clade diversification in Costa Rican orchids. *Evolution* 66(10):3035–3052.
- Coyne JA, Orr HA (2004) *Speciation* (Sinauer, Sunderland, MA).
- Rabosky DL, McCune AR (2010) Reinventing species selection with molecular phylogenies. *Trends Ecol Evol* 25(2):68–74.
- Jablonski D (2008) Species selection: Theory and data. *Annu Rev Ecol Syst* 39:501–524.
- Dobzhansky TH (1937) *Genetics and the Origin of Species* (Columbia Univ Press, New York).
- Mayr E (1963) *Animal Species and Evolution* (Belknap Press Harvard Univ Press, Boston).
- Nosil P, Schluter D (2011) The genes underlying the process of speciation. *Trends Ecol Evol* 26(4):160–167.
- Sobel JM, Chen GF, Watt LR, Schemske DW (2010) The biology of speciation. *Evolution* 64(2):295–315.
- Turelli M, Barton NH, Coyne JA (2001) Theory and speciation. *Trends Ecol Evol* 16(7):330–343.
- Matute DR, Butler IA, Turissini DA, Coyne JA (2010) A test of the snowball theory for the rate of evolution of hybrid incompatibilities. *Science* 329(5998):1518–1521.
- Maheshwari S, Barbash DA (2011) The genetics of hybrid incompatibilities. *Annu Rev Genet* 45:331–355.
- Wiens JJ, Engstrom TN, Chippindale PT (2006) Rapid diversification, incomplete isolation, and the “speciation clock” in North American salamanders (Genus Plethodon): Testing the hybrid swarm hypothesis of rapid radiation. *Evolution* 60(12):2585–2603.
- Allmon WD (1992) A causal analysis of the stages in allopatric speciation. *Oxford Surveys in Evolutionary Biology*, eds Futuyma DJ, Antonovics J (Oxford Univ Press, Oxford), pp 219–257.
- Ricklefs RE, Bermingham E (2007) The causes of evolutionary radiations in archipelagoes: Passerine birds in the lesser antilles. *Am Nat* 169(3):285–297.
- Stanley SM (1979) *Macroevolution: Pattern and Process* (Freeman, San Francisco).
- Wiens JJ (2004) What is speciation and how should we study it? *Am Nat* 163(6):914–923.
- Price TD (2010) The roles of time and ecology in the continental radiation of the Old World leaf warblers (Phylloscopus and Seicercus). *Philos Trans R Soc Lond B Biol Sci* 365(1547):1749–1762.
- Weir JT, Price TD (2011) Limits to speciation inferred from times to secondary sympatry and ages of hybridizing species along a latitudinal gradient. *Am Nat* 177(4):462–469.
- Rosenblum EB, et al. (2012) Goldilocks meets Santa Rosalia: An ephemeral speciation model explains patterns of diversification across time scales. *Evol Biol* 39(2):255–261.
- Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO (2012) The global diversity of birds in space and time. *Nature* 491(7424):444–448.
- Rabosky DL, et al. (2013) Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nat Commun* 4:1958, 10.1038/ncomms2958.
- Hodges SA (1997) Floral nectar spurs and diversification. *Int J Plant Sci* 158:581–588.
- Panhuis TM, Butlin R, Zuk M, Tregenza T (2001) Sexual selection and speciation. *Trends Ecol Evol* 16(7):364–371.
- Yukilevich R (2012) Asymmetrical patterns of speciation uniquely support reinforcement in *Drosophila*. *Evolution* 66(5):1430–1446.
- Gray AP (1958) *Bird Hybrids, A Check-List with Bibliography* (Commonwealth Agricultural Bureaux, Farnham, UK).
- Price TD, Bouvier MM (2002) The evolution of F1 postzygotic incompatibilities in birds. *Evolution* 56(10):2083–2089.
- Moyle LC, Nakazato T (2010) Hybrid incompatibility “snowballs” between *Solanum* species. *Science* 329(5998):1521–1523.
- Wang RJ, Ane C, Payseur BA (2013) The evolution of hybrid incompatibilities along a phylogeny. *Evolution*, 10.1111/evo.12173.
- Servedio MR, Noor MAF (2003) The role of reinforcement in speciation: theory and data. *Annu Rev Ecol Syst* 34:339–364.
- Raftery AE, Newton MA, Satagopan JM, Krivitsky PN (2007) Estimating the integrated likelihood via posterior simulation using the harmonic mean identity. *Bayesian Statistics*, eds Bernardo JM, Bayarri MJ, Berger JO, Dawid AP (Oxford Univ Press, Oxford), pp 1–45.
- Mallet J (2007) Species, concepts of. *Encyclopedia of Biodiversity*, ed Levin SA (Elsevier, Amsterdam).
- Rabosky DL, Lovette IJ (2008) Density-dependent diversification in North American wood warblers. *Proc Biol Sci* 275(1649):2363–2371.
- Sepkoski JJ (1978) A kinetic model of Phanerozoic taxonomic diversity I. Analysis of marine orders. *Paleobiology* 4:223–251.
- Rabosky DL (2010) Primary controls on species richness in higher taxa. *Syst Biol* 59(6):634–645.
- Grafen A (1989) The phylogenetic regression. *Philos Trans R Soc Lond B Biol Sci* 326(1233):119–157.
- Langear R, Ho SYW, Love D, Bromham L (2010) Mutation rate is linked to diversification in birds. *Proc Natl Acad Sci USA* 107(47):20423–20428.
- Price T (2008) *Speciation in Birds* (Roberts and Company, Greenwood Village, CO).
- Matute DR, Coyne JA (2010) Intrinsic reproductive isolation between two sister species of *Drosophila*. *Evolution* 64(4):903–920.
- Gavriliets S (2004) *Fitness Landscapes and the Origin of Species* (Princeton Univ Press, Princeton).
- Wu C-I, Ting C-T (2004) Genes and speciation. *Nat Rev Genet* 5(2):114–122.
- Qvarnström A, Rice AM, Ellegren H (2010) Speciation in *Ficedula* flycatchers. *Philos Trans R Soc Lond B Biol Sci* 365(1547):1841–1852.
- Grant PR, Grant BR (1997) Genetics and the origin of bird species. *Proc Natl Acad Sci USA* 94(15):7768–7775.
- Darwin C (1859) *On the Origin of Species by Means of Natural Selection* (John Murray, London).
- Pfennig DW, Pfennig KS (2013) *Evolution's Wedge: Competition and the Origins of Diversity* (Univ California Press, Berkeley).
- Reznick DN, Ricklefs RE (2009) Darwin's bridge between microevolution and macroevolution. *Nature* 457(7231):837–842.
- McKinnon JS, Rundle HD (2002) Speciation in nature: the threespine stickleback model systems. *Trends Ecol Evol* 17:480–488.
- Pigot AL, Tobias JA (2013) Species interactions constrain geographic range expansion over evolutionary time. *Ecol Lett* 16(3):330–338.
- Ricklefs RE (2010) Host-pathogen coevolution, secondary sympatry and species diversification. *Philos Trans R Soc Lond B Biol Sci* 365(1543):1139–1147.
- Ezard THG, Pearson PN, Aze T, Purvis A (2012) The meaning of birth and death (in macroevolutionary birth-death models). *Biol Lett* 8(1):139–142.
- Coyne JA, Orr HA (1989) Patterns of speciation in *Drosophila*. *Evolution* 43:362–381.
- Mendelson TC (2003) Sexual isolation evolves faster than hybrid inviability in a diverse and sexually dimorphic genus of fish (Percidae: Etheostoma). *Evolution* 57(2):317–327.
- Stelkens RB, Young KA, Seehausen O (2010) The accumulation of reproductive incompatibilities in African cichlid fish. *Evolution* 64(3):617–633.
- van der Linde K, Houle D, Spicer GS, Stepan SJ (2010) A supermatrix-based molecular phylogeny of the family Drosophilidae. *Genet Res* 92(1):25–38.
- Nosil P (2013) Degree of sympatry affects reinforcement in *Drosophila*. *Evolution* 67(3):868–872.
- Magallón S, Sanderson MJ (2001) Absolute diversification rates in angiosperm clades. *Evolution* 55(9):1762–1780.