

patibilities, and the results for all the model comparisons are shown in Fig. 3. These data show that the number of incompatibilities does not accumulate linearly with divergence time and is more consistent with a quadratic increase, as expected if the evolution of those incompatibilities obeys the snowball effect. This result holds regardless of which estimate of divergence time we use (Fig. 1 and figs. S1 to S3). We thus conclude that hybrid incompatibilities between these species accumulate substantially faster than linearly with respect to their divergence time.

In addition to fitting the linear and quadratic models to regions that caused a relative viability lower than 0.5, we used more stringent criteria for inviability, fitting the two models to regions that showed relative viabilities lower than 0.3, 0.1, and 0. The results were similar in all cases (Fig. 1 and figs. S1 to S3) and revealed that, regardless of their size, deleterious epistatic interactions accumulate faster than linearly with divergence time.

Besides counting incompatibilities, our results can be used to identify and isolate genes causing reproductive isolation between species. Deletion mapping in *D. melanogaster*/*D. simulans* hybrids has identified two “hybrid inviability” genes with

function in nuclear transport and whose divergence occurred via natural selection (6, 8, 22).

By confirming a key prediction of the DM theory and showing a snowball effect of the accumulation of genetic incompatibilities causing reproductive isolation, we support the view that postzygotic reproductive isolation often results from deleterious interactions in hybrids between genes that behave normally within species.

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#### Supporting Online Material

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## Hybrid Incompatibility “Snowballs” Between *Solanum* Species

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Among the reproductive barriers that can isolate species, hybrid sterility is frequently due to dysfunctional interactions between loci that accumulate between differentiating lineages. Theory describing the evolution of these incompatibilities has generated the prediction, still empirically untested, that loci underlying hybrid incompatibility should accumulate faster than linearly with time—the “snowball effect.” We evaluated the accumulation of quantitative trait loci (QTL) between species in the plant group *Solanum* and found evidence for a faster-than-linear accumulation of hybrid seed sterility QTL, thus empirically evaluating and confirming this theoretical prediction. In comparison, loci underlying traits unrelated to hybrid sterility show no evidence for an accelerating rate of accumulation between species.

The Dobzhansky-Muller model of hybrid incompatibility [after (1, 2)] proposes that hybrid sterility and inviability are due to negative genetic interactions between two or more loci [commonly called “Dobzhansky-Muller incompatibilities” (DMIs)] that have accumulated substitutions in diverging lineages. When brought together in hybrids, alleles in each divergent lineage interact dysfunctionally, which results in reduced hybrid fitness (3). The action of DMIs is supported by empirical observation of the segregation of sterility in recombinant populations, and the molecular genetic

description of individual interacting loci underlying hybrid incompatibility phenotypes (4, 5). The Dobzhansky-Muller model (3, 6–9) has produced empirically testable predictions including the “snowball effect”—the number of DMIs accumulating between lineages is expected to “snowball” (increase faster than linearly) with increasing time since lineage divergence (3, 6). Formally, because DMIs are due to gene interactions (epistasis), the number of expected DMIs increases with the square of the number of substitutions differentiating two lineages, when DMIs are due to pairwise epistasis; DMIs due to interactions among more than two loci are expected to accumulate even faster (6). Previous attempts to detect the snowball effect by measuring the strength of reproductive isolation between lineages, rather than the number of genes involved, have failed to find a greater-than-linear

increase in sterility over time (10–12). However, testing this theoretical prediction requires information on the number of DMIs contributing to specific isolating barriers among multiple closely related species, rather than simply their phenotypic effects on hybrid sterility (3, 6).

To evaluate the expected snowball of DMIs, we used data from three quantitative trait loci (QTL) mapping experiments among species in the plant genus *Solanum* (13–15). Each QTL experiment used a unique library of hybrid introgression lines [near-isogenic lines (NILs)] in which all or most of the genome of a wild (undomesticated) *Solanum* species (*Solanum pennellii*, *Solanum habrochaites*, or *Solanum lycopersicoides*) was represented as short individual chromosomal regions serially introgressed into the genetic background of domesticated tomato (*Solanum lycopersicum*). These three experiments are comparable in the mean and distribution of hetero-specific introgression sizes and the generations of crossing used to create the lines, and they have similar statistical power for detecting pollen and seed sterility QTL (Table 1) (15). Each experiment identified the number, genomic location, and phenotypic effect—size of chromosomal regions associated with two separate postzygotic sterility phenotypes (pollen sterility and seed sterility) acting between two species (15). In each population, we also analyzed morphological traits unrelated to hybrid sterility (fruit shape and size of fertile seeds) as an internal control. As a proxy for time since lineage splitting, we estimated pairwise species molecular divergence as the number of synonymous substitutions per synonymous site ( $K_s$ ) at six unlinked loci distributed throughout the genome (15).

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Although we identified the signal of a snowball effect, it was significant only for seed sterility. The failure to detect a snowball for pollen sterility could be due to several, nonexclusive contributing factors, which we currently cannot differentiate (15). For example, DMIs for pollen sterility might accumulate noncombinatorially [unlike in Orr's (3) original snowball model], which could result in a more linear relation between the number of DMIs and the time since divergence. This could occur, for example, if substitutions contributing to pollen sterility DMIs over time were not independent of each other, as might happen if isolating barriers evolve as the result of genetic conflicts (17). Alternatively, an attenuated snowball for pollen sterility could be due to bias against QTL detection in the cross with the greatest genetic distance, and/or rate heterogeneity in the accumulation of sterility loci (15). Finally, if there is a systematic difference in the complexity of genetic interactions underlying male versus female hybrid sterility, we may have differing abilities to detect them with our introgression line approach, which can detect DMIs due to pairwise epistatic interactions but not DMIs requiring more than one chromosomal segment from each species, i.e., multilocus interactions (4, 15). Current data, although sparse, suggest that DMIs responsible for male sterility might frequently be due to complex multilocus interactions (18–20). In *Solanum*, hybrid introgression lines show progressively more pollen sterility depending on whether they carry one, two, or three conspecific introgressions from the donor species, which suggests that multilocus interac-

tions are necessary for the expression of some hybrid pollen sterility (21); there is no equivalent increase in seed sterility with number of conspecific introgressions.

Overall, our results indicate that the accumulation of sterility loci follows a different trajectory from the accumulation of loci for other quantitative species differences (Fig. 1), consistent with the unique genetic basis expected to underpin species reproductive isolating barriers. Our analysis examines the accumulation of loci contributing to individually evaluated hybrid sterility traits (pollen sterility and seed sterility); therefore, we explicitly evaluate the accumulation of genetic loci rather than sterility phenotypes, and we do not conflate the accumulation of loci underlying genetically and developmentally distinct isolating barriers [for example, by examining the accumulation of both male and female sterility as a single “total isolation” phenotype (12)]. In doing so, we uncover direct empirical support for the Dobzhansky-Muller model of hybrid incompatibility, and the snowball prediction in particular.

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## The Ecological Significance of Tool Use in New Caledonian Crows

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Tool use is so rare in the animal kingdom that its evolutionary origins cannot be traced with comparative analyses. Valuable insights can be gained from investigating the ecological context and adaptive significance of tool use under contemporary conditions, but obtaining robust observational data is challenging. We assayed individual-level tool-use dependence in wild New Caledonian crows by analyzing stable isotope profiles of the birds' feathers, blood, and putative food sources. Bayesian diet-mixing models revealed that a substantial amount of the crows' protein and lipid intake comes from prey obtained with stick tools—wood-boring beetle larvae. Our calculations provide estimates of larva-intake rates and show that just a few larvae can satisfy a crow's daily energy requirements, highlighting the substantial rewards available to competent tool users.

New Caledonian crows (*Corvus moneduloides*) (hereafter NC crows) use tools for extractive foraging (1, 2). Controlled experiments with captive-bred, juvenile NC crows have recently shown that basic stick tool use is heritable, and hence an evolved adaptation (3). Although we can only speculate about the selective pressures that fostered the evolution of

tool use in NC crows, it is possible to investigate quantitatively how this behavior is maintained under contemporary ecological conditions.

Very little is currently known about the general foraging ecology of NC crows, or the ecological context of their tool use, because of the difficulty of observing wild, free-ranging subjects in their tropical habitats (4, 5). However, long-

term fieldwork has revealed that NC crows exploit two potentially nutritious food sources provided by candlenut trees (*Aleurites moluccana*; Euphorbiaceae): nuts and wood-boring longhorn beetle larvae (*Agrianome fairmairei*; Cerambycidae). In areas where these trees grow, NC crows crack their nuts by dropping them onto hard surfaces (6), and they use stick tools to probe for larvae in decaying, beetle-infested trunks (5, 7). The larva-extraction technique of NC crows relies on exploiting defensive responses of their prey, similar to the well-known “termite fishing” of chimpanzees (8). Crows insert a twig or leaf stem into a burrow, “teasing” the larva by repeatedly poking it with the tool until it bites the tip of the tool with its powerful mandibles and can be levered out (fig. S1 and

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