Single origin of Malagasy Carnivora from an African ancestor

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The Carnivora are one of only four orders of terrestrial mammals living in Madagascar today. All four (carnivorans, primates, rodents and lipotyphlan insectivores) are placental mammals with limited means for dispersal, yet they occur on a large island that has been surrounded by a formidable oceanic barrier for at least 88 million years[1,2], predating the age of origin for any of these groups[3,4]. Even so, as many as four colonizations of Madagascar have been proposed for the Carnivora alone[5]. The mystery of the island's mammalian origins is confounded by its poor Tertiary fossil record, which leaves us with no direct means for estimating dates of initial diversification. Here we use a multigene phylogenetic analysis to show that Malagasy carnivorans are monophyletic and thus the product of a single colonization of Madagascar by an African ancestor. Furthermore, a bayesian analysis[6] of divergence ages for Malagasy carnivorans and lemuriforms indicates that their respective colonizations were temporally separated by tens of millions of years. We therefore conclude that a single event, such as vicariance or common dispersal, cannot explain the presence of both groups in Madagascar.

Madagascar is an island of remarkable faunal and floral diversity where species endemism reaches 100% in some animal groups. It is also unusual for its endemism at high taxonomic levels and skewed representation of many biotic groups compared with continental communities. Krause *et al.*[3] have referred to these patterns as Madagascar's unique signature of 'imbalance and endemism'. This signature undoubtedly derives from the island's long history of complete isolation from other landmasses. Madagascar, and other fragments of the Gondwanan supercontinent, split from mainland Africa nearly 165 million years (Myr) ago and began its southeastward trajectory to reach its current position at approximately 121 Myr ago (ref. 1). For this entire period, and the subsequent 30 Myr or so, it remained attached to India, forming the Indo-Madagascar continent. These two landmasses finally separated approximately 88 Myr ago (ref. 2) when India started its northward movement to subsequently collide with the Asian continent in the Early Eocene[7]. Thus, although Madagascar had a long association with India in particular, and remains within 400 km of Africa, it has otherwise been completely isolated and surrounded by a deep and wide oceanic barrier for at least the past 88 Myr. Although it has been proposed that a nearly continuous landbridge linked Madagascar to Africa for much of the Cenozoic[8], the geologic data on which this hypothesis is based are extremely frail[9].

The various biogeographical mechanisms that could explain the presence of mammals and other terrestrial vertebrates in Madagascar are: Gondwanan vicariance[10]; land-mediated dispersal (for example, landbridge connections)[8]; and 'sweepstakes' over-water dispersal (that is, 'rafting')[11,12] or island-hopping[3]. Each model is compatible with contrasting phylogenetic and temporal patterns. The Gondwanan vicariance mechanism implies ancient divergence dates, with all clades of that antiquity having equally probable chances of occurrence in Madagascar. The landbridge model[8] implies divergence dates constrained within a period from the Middle Eocene epoch (~45 Myr ago) until the Early Miocene epoch (~26 Myr ago), also with equally probable opportunities for clade representation. The sweepstakes dispersal model, however, implies that divergence dates should be more randomly distributed within the time frame of placental evolution, and that some groups would be more suited to withstand over-water dispersal than others. Ascertaining which of these mechanisms appears to have been the most likely requires determining both the phylogeny and the age of Malagasy lineages[8].

Using maximum likelihood, minimum evolution, maximum parsimony and bayesian analysis, we phylogenetically analysed the Malagasy Carnivora and related feliform and caniform outgroups by sampling four genes from three unlinked genetic loci. The non-carnivoran outgroups, human (*Homo sapiens*) and mole (*Scalopus aquaticus*), were included to ensure proper rooting of the phylogeny. Several notable results emerge from the phylogenetic analysis, regardless of the optimality criteria used. The Malagasy carnivorans are shown to constitute a clade with robust statistical support (Fig. 1), which is most relevant for testing the validity of the alternative biogeographical models. This result indicates that the

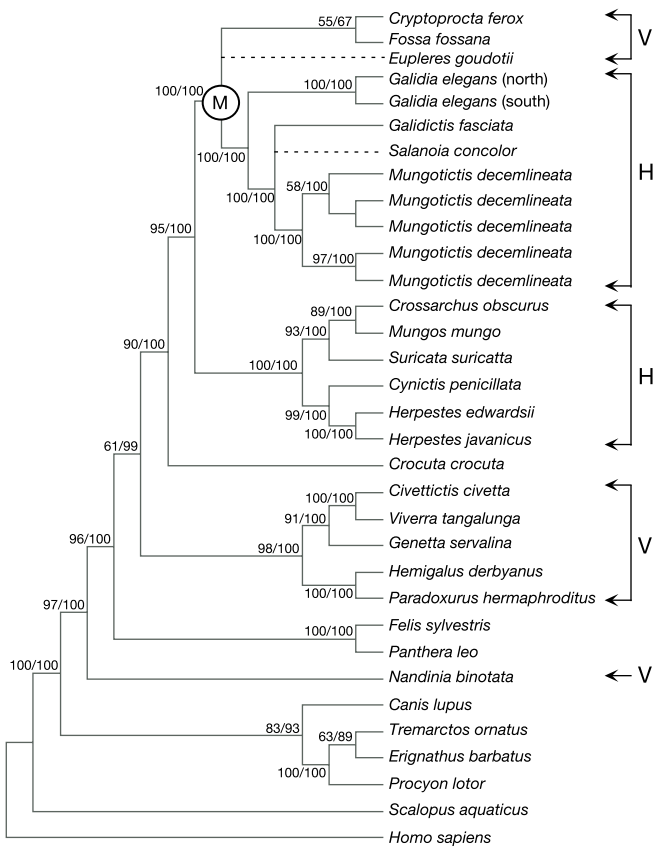

Figure 1 Maximum likelihood phylogeny of Malagasy Carnivora and selected carnivoran outgroups. The tree is rooted with mole (*Scalopus aquaticus*) and human (*Homo sapiens*). The tree topology has a likelihood score of 38580.775. The dashed lines indicate taxa sampled from museum specimens for which data could only be obtained from the *cytochrome b* gene because of poor DNA quality. The tree otherwise represents an analysis wherein the four data sets were combined in a single analysis. Numbers on nodes represent statistical support from a 'fast' bootstrap analysis under the likelihood criterion (first number) and posterior probabilities from the bayesian analysis (second number). Circled M, Malagasy carnivoran node; V, taxa previously classified within the family Viverridae; H, within the family Herpestidae. (See also Table in Supplementary Information.)

Malagasy Carnivora are the product of a single colonization of Madagascar, a conclusion that is consistent with phylogenetic analyses of the other three groups of endemic modern terrestrial mammals in Madagascar[13–15]. Within the Malagasy Carnivora clade, *Cryptoprocta* and *Fossa* appear to be basal (although their relative relationships within the Malagasy clade are poorly resolved, as indicated by weak statistical support and/or differing resolutions depending on optimality criteria) and the mongoose-like lineages (*Galidia*, *Galidictis* and *Mungotictis*) are shown to comprise a well-supported internal clade. Cytochrome *b* data from museum skins of *Salanoia* and *Eupleres* indicate that they too lie within the Malagasy carnivoran clade, with *Eupleres* falling in an unresolved basal position and *Salanoia* belonging to the Malagasy 'mongoose' subclade. A clade comprising African and Asian Herpestidae is found by all optimality criteria to be the nearest outgroup of the Malagasy clade, with the African Hyaenidae as sister to this combined clade. Together, these results clearly indicate that the Malagasy carnivorans, including the large and very puma-like *Cryptoprocta*[16], are most closely related to the Herpestidae (in the strict sense), which are optimized to be of African origin (Fig. 2). In the higher-level systematics of the Carnivora, the Malagasy clade lies deeply nested within a feliform clade with the 'viverrid' *Nandinia* as the basal feliform lineage. The Malagasy clade does not, however, belong to a monophyletic Viverridae, nor do subsets of Malagasy taxa nest within the Herpestidae or Felidae, as has been previously postulated[5]. Instead, our results further confirm that the traditional family Viverridae is paraphyletic[17] and, contrary to long-held beliefs, does not contain any Malagasy species.

Given that the hypothesis of a single origin from an African ancestor for Malagasy carnivorans is precisely congruent with similar phylogeographical observations of Malagasy Primates (Lemuriformes)[15,18], it becomes informative to investigate the relative divergence times of the two groups. If a single biogeographical event, such as vicariance or common dispersal, were to explain the presence of both groups in Madagascar, we would expect to see similar (if not identical) age estimates for both. We used bayesian methods[6] to estimate divergence ages for the Malagasy carnivorans and lemuriforms. These methods do not assume a molecular clock and also allow for several fossil-based calibrations to be used simultaneously in a given analysis. Dates for the Malagasy Carnivora were estimated individually for the four genes sampled by this study and for a data set in which the four genes were combined. For the *interphotoreceptor retinoid-binding protein* (*IRBP*) and *cytochrome b* data sets, and for their combination, an extensive sample of lemuriform taxa was available, allowing a simultaneous analysis of the two groups. This latter comparison is important because it uses homologous data and identical methods of analysis for two independent clades of Madagascar's colonizing mammals. Consequently, even if there is error in the determination of the absolute age of the two clades, the determination of relative ages should be similarly biased and therefore directly comparable.

Visual inspection of the phylogeny used for the comparative age analysis (Fig. 3), wherein branches are drawn to be proportional to estimated branch lengths, shows an obvious differential in clade depth between the two groups. This differential is clarified by the bayesian analysis, which indicates that the two Malagasy clades are

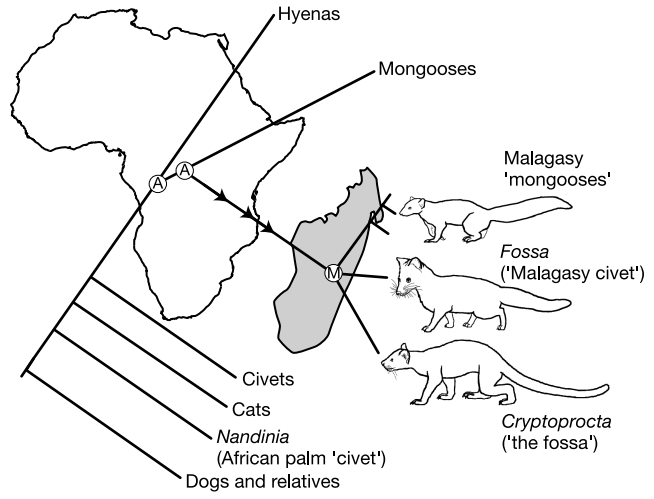

Figure 2 Proposed biogeographical model of carnivoran dispersal from Africa. The figure illustrates single colonization of Madagascar and subsequent radiation into extant lineages. A, African radiation; M, Malagasy radiation. Malagasy radiation is illustrated as a trifurcation because of the poor resolution (that is, low statistical support and/or conflicting placements) of *Cyptoprocta* and *Fossa* with respect to the Malagasy 'mongoose' clade. The placement of *Salanoia* and *Eupleres* is not illustrated or implied because of limited data for these taxa. (Figure prepared by M. Hill Donnelly, Field Museum of Natural History.)

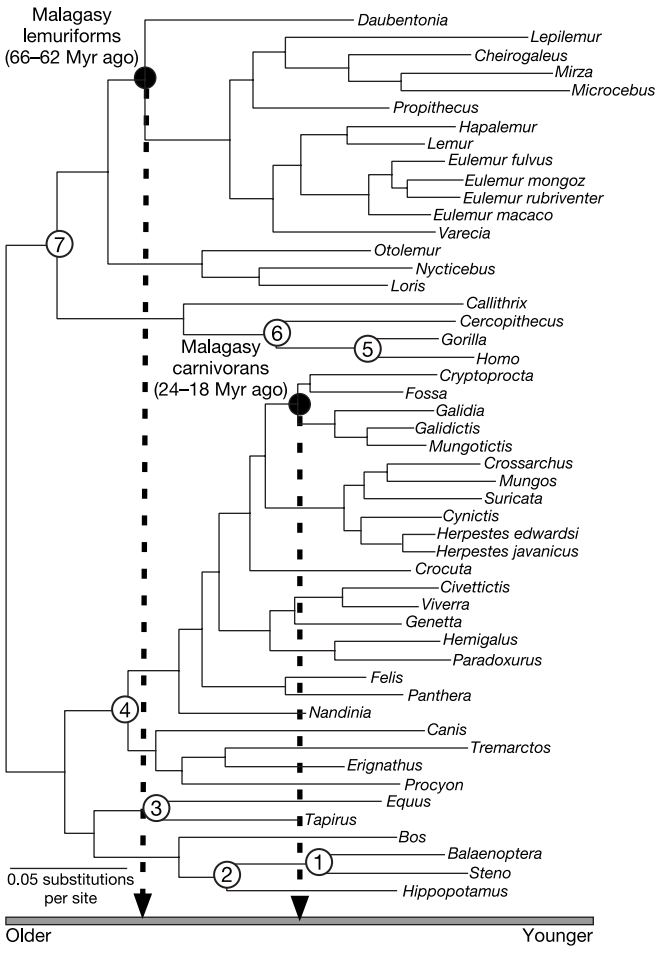

Figure 3 Comparative age analysis of Lemuriformes and Malagasy Carnivora. Branch lengths from combined IRBP and cytochrome *b* tree are drawn to be proportional with estimated rates of change per site as calculated by *estbranches*. The tree was rooted with *Didelphis virginiana* and *Mus musculus* (not shown). The time bar at the bottom of the figure is not meant to imply a clock-like tree. Numbered nodes indicate calibration points in the bayesian analysis. These are: (1) toothed whale/baleen whale divergence 33–40 Myr ago; (2) whale/hippo divergence 51–60 Myr ago; (3) horse/tapir (or rhinoceros for *ND2*) divergence 50–58 Myr ago; (4) caniform/feliform divergence 45–65 Myr ago; (5) human/gorilla divergence 8–12 Myr ago, (6) monkey/ape divergence 32–38 Myr ago; (7) basal primate radiation 63–90 Myr ago. Owing to poor outgroup sequence availability for transthyretin, only calibrations 4, 5 and 6 were used in the analysis of that gene, and calibration 4 in the four-gene combined analysis. See Supplementary Information for relevant literature citations.

Table 1 **Bayesian estimates of geologic age by data set**

Data set	Malagasy Carnivora	Malagasy Primates
Cytochrome b (1,140 bp)	23 Myr ago (15–32)	64 Myr ago (50–78)
IRBP (exon I, 945 bp)	20 Myr ago (11–31)	62 Myr ago (47–75)
ND2 (1044 bp)	24 Myr ago (16–33)	NA
Transthyretin (intron I, 897 bp)	18 Myr ago (11–25)	NA
Cytochrome b and *IRBP*	24 Myr ago (18–31)	66 Myr ago (55–75)
Four genes combined	20 Myr ago (15–26)	NA

See Fig. 3 legend for description of fossil calibrations used for the estimation of geologic ages. Numbers in parentheses represent 95% credibility intervals. NA, not available.

of considerably different ages (Table 1). The range of posterior mean estimates for the Malagasy carnivorans lies between 24 and 18 Myr ago, whereas that for the lemuriforms lies between 66 and 62 Myr ago, suggesting that their relative colonizations were separated by 38 Myr. Even when one examines the extreme boundaries of the 95% credibility intervals (95% CI) for the two groups, the absolute minimum time separating their relative divergence ages is 14 Myr (or, likewise, as many as 67 Myr). Most importantly, in any of the possible comparisons, the disparity in age estimates indicates that the same biogeographical event cannot explain the joint appearance of both groups in Madagascar.

What then are the biogeographical implications of this temporal analysis? If the presence of either or both groups were to be explained by Indo-Madagascar vicariance, we would expect to see substantially more ancient divergence ages (namely, >88 Myr), with each group having its closest relatives in Indo-Asia. We would also expect to see representation of closely related groups (for example, other feliforms, caniform carnivorans, lorisiform primates) in Madagascar. This is not what we observe. Furthermore, the fact that both Malagasy clades appear to have origins in the Tertiary period is compatible with the known fossil record of Madagascar. The only mammals known from the Late Cretaceous period of Madagascar are multituberculates, gondwanatheres and marsupials[19,20], all of which are now extinct on the island. Conversely, colonization of Madagascar via the hypothesized landbridge would imply posterior mean-age estimates that were approximately equivalent in the two groups, with ages for both falling within the proposed temporal window. Moreover, we would expect to see greater representation of both closely and distantly related groups of mammals which also would have found dispersal across a continuous landbridge to be expedient. Again, these are not the observed patterns. Instead, the data are most compatible with a model of over-water dispersal from African ancestry. All of the posterior mean-age estimates for both clades are entirely outside the 45–26 Myr ago temporal window hypothesized for the putative landbridge (Fig. 4), which is actually rather remarkable given that this window spans more than one-third of the Cenozoic era. Although the extreme lower bounds of the 95% CIs for the carnivorans overlap slightly with the temporal window, the consistent estimation of posterior means between 18–24 Myr ago from four different genes suggests that they are a good approximation of actual divergence age, and all post-date the entire span of the putative landbridge. Finally, it is notable that the 95% CIs are reduced in both of the combined data analyses, and in the four-gene combination for the carnivorans, do not overlap with the landbridge temporal window.

As of late 2001, the total known extant native terrestrial mammal fauna of Madagascar comprises 101 species (ref. 21) belonging to four independent clades (rodents[13], lipotyphlans[14], lemurs[15] and carnivorans—herein). Thus, the entire extant land mammal fauna of the island can be explained by only four colonization events, and at least for the two clades compared here, their immigrations appear to have occurred at random as predicted by Simpson's 'sweepstakes' model of over-water dispersal[12]. It is significant to note that for at least three of the four founding clades of terrestrial mammals, some

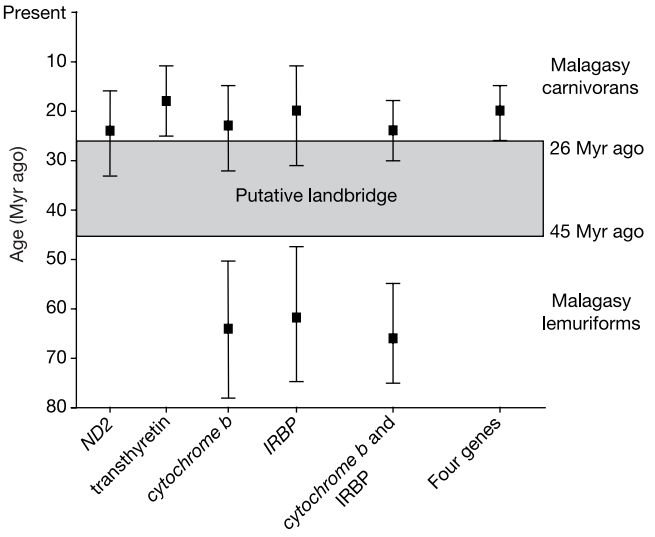

Figure 4 Comparison of bayesian age estimates for Malagasy carnivorans (above) and lemuriforms (below) relative to proposed landbridge temporal window[8]. Filled squares, posterior mean estimates of clade divergence ages in Madagascar; thin lines, 95% credibility intervals for age estimates.

species have ecophysiological specializations, such as the ability to hibernate or go into torpor[22,23], which would have predisposed them for enduring long periods of drought and food shortage[11,24,25]. If these specializations are primitive within each group, as has been concluded for the tenrecs[14], the over-water dispersal model gains yet more credence. Given the morphological and ecological diversity of the island's existing native mammals, this further underscores that the successful crossing of a large water barrier, with subsequent colonization and diversification, has been a very rare event indeed among non-volant mammals. This pattern makes for a comparison with that observed for chameleons, wherein over-water dispersal has also been posited as the likely mechanism explaining their biogeographical distribution[26]. In this group, however, 'sweepstakes' dispersal has been inferred to have been a relatively common event, in contrast to the extreme rarity here reported for terrestrial mammals. □

Methods

Two mitochondrial genes, *cytochrome b* and *ND2*, were sequenced in their entirety, yielding alignments of 1,140 base pairs (bp) and 1,044 bp, respectively. Two nuclear genes were partly sequenced: exon 1 of the *interphotoreceptor retinoid-binding protein* (*IRBP*), and intron 1 of the *transthyretin* gene, yielding alignments of 945 bp and 897 bp, respectively. In humans, *transthyretin* is located on chromosome 18 and *IRBP* on chromosome 10; we thus assume that they are also independently segregating in Carnivora. All sequences were aligned by eye, owing to a lack of indels, except for the *transthyretin* sequences. For this gene, sequences were aligned with Clustal W (1.4) and adjusted manually. Before phylogenetic analysis, all large taxon-specific indels were omitted. Phylogenetic analysis was done on a combined data set of the four genes with PAUP* version 4.0b6 (Altivec)[27] by using the maximum parsimony, minimum evolution and maximum likelihood optimality criteria. Settings were as follows: maximum parsimony with starting trees obtained by stepwise addition, minimum evolution with an HKY85 correction and starting trees obtained via neighbour-joining, and maximum likelihood with a GTR + Γ + I model; all other settings were set by default. To assess statistical support for hypothesized clades, bootstrap analysis was done with 100 bootstrap replicates sampling 10 replicates of the random stepwise addition option; 100 replicates of the 'fast' bootstrap algorithm was used for the maximum likelihood analysis. Sequences were also analysed with a Markov chain Monte Carlo approach using the program MrBayes version 2.01[28] with clade support assessed by posterior probability. Four Markov chains, one cold and three heated, were allowed to run for 1,000,000 generations using random starting trees. The model used was equivalent to a GTR + Γ likelihood model. MacClade 4.0 PPC[29] was used to optimize geographical origins for internal nodes within the feliform carnivorans.

Appropriate likelihood models for the bayesian analysis of divergence ages were determined using the program MODELTEST version 3.06 PPC[30] with the mean rate for each category of the discrete approximation of the gamma distribution estimated with PAUP*. The program *estbranches* was used to estimate branch lengths for the *IRBP*,

cytochrome b, *ND2* and *transthyretin* genes individually and in various combinations (see text). We used the program *divtime5bmac* to estimate divergence times, using the output from *estbranches* (both programs are available from J. Thorne). Markov chain Monte Carlo analyses were run for 1 million generations after a burnin of 100,000 generations. Chains were sampled every 100 generations, yielding 10,000 samples of the Markov chain. Seven independent calibrations were used specifying upper and lower bounds (see Fig. 3). For each analysis, one approximation of the prior distribution and at least two separate approximations of the posterior distribution were obtained. Comparison of the prior and posterior approximations reveals the impact of the sequence data on the divergence time estimates. The separate approximations of the posterior distributions were done by starting the Markov chain Monte Carlo technique from different initial states. The similarity of these approximations indicates that the Markov chains have successfully converged to the posterior distribution.

Sequences are deposited in GenBank under accession numbers AY170012–AY170116, AY187007, and AY187008. Sequence alignments are available in Supplementary Information.

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The effect of aggressiveness on the population dynamics of a territorial bird

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A central issue in ecology lies in identifying the importance of resources, natural enemies and behaviour in the regulation of animal populations. Much of the debate on this subject has focused on animals that show cyclic fluctuations in abundance[1–7]. However, there is still disagreement about the role of extrinsic (food, parasites or predators) and intrinsic (behaviour) factors in causing cycles[2,8–10]. Recent studies have examined the impact of natural enemies[1,3,4,7], although spatial patterns resulting from restricted dispersal or recruitment are increasingly recognized as having the potential to influence unstable population dynamics[5,6,11–13]. We tested the hypothesis that population cycles in a territorial bird, red grouse *Lagopus lagopus scoticus*, are caused by delayed density-dependent changes in the aggressiveness and spacing behaviour of males. Here we show that increasing aggressiveness experimentally for a short period in autumn reduced recruitment and subsequent breeding density by 50%, and changed population trajectories from increasing to declining. Intrinsic processes can therefore have fundamental effects on population dynamics.

We examined the role of intrinsic processes in the population dynamics of *Lagopus lagopus scoticus*. According to the 'territorial behaviour' hypothesis, the population cycles of this species are caused by changes in aggressiveness that influence the recruitment rate of young males in autumn[14–16]. Aggressiveness limits density by affecting territory size[17], and shows delayed density-dependence, with a peak during the first year of a population decline[15,16]. The mechanism proposed for this time lag is based on changes in kinship and differential aggressive behaviour towards kin and non-kin[14,15,18,19]. We designed an experiment to test the effect of aggressiveness on red grouse population dynamics. Our aim was to put grouse populations at the stage in the cycle when aggressiveness is higher than expected for the prevailing density[15], and to test the prediction that this would cause a population decline. We used testosterone implants to increase the aggressiveness of old (territorial) males in four populations for three months during autumn. We specifically predicted that, relative to control populations, 'aggres-

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