



International Conservation Policy Delivers Benefits for Birds in Europe

Paul F. Donald, *et al.*
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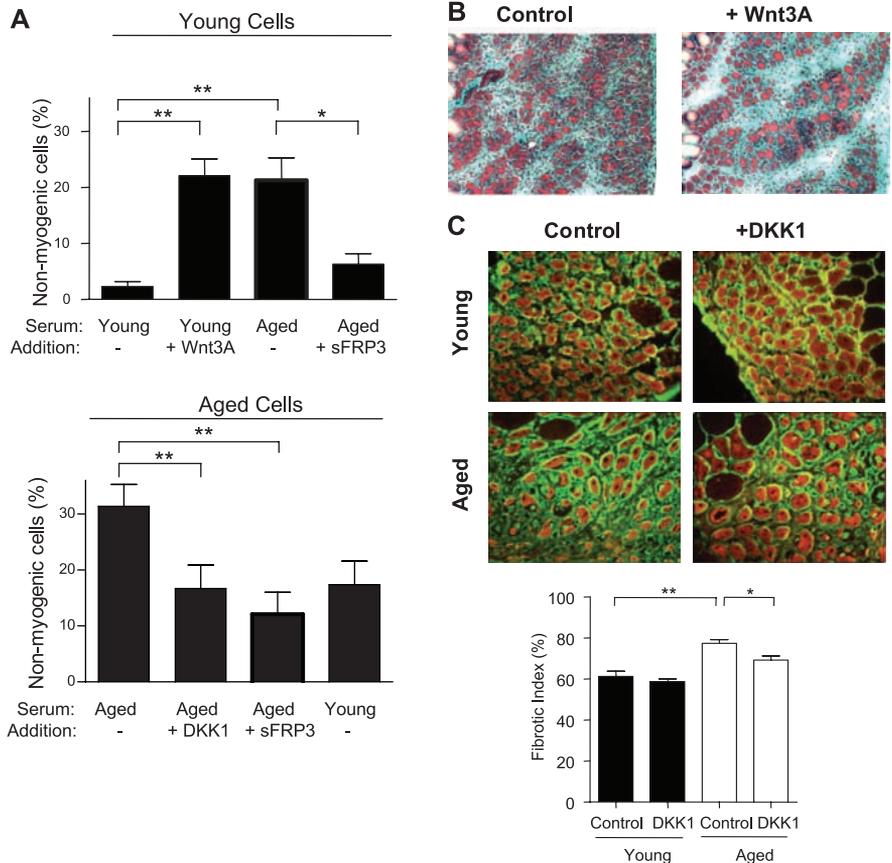
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Fig. 4. The effects of the aged environment on myogenic progenitor cell fate and muscle regeneration are mediated by the Wnt signaling pathway. **(A)** (Top) The fate of myogenic progenitors from young mice incubated in young serum, with or without exogenous Wnt3A, or incubated in aged serum, with or without the Wnt inhibitor sFRP3, for 1½ days. (Bottom) Fate of myogenic progenitors from aged mice incubated in young serum or in aged serum, with or without sFRP3 or DKK1, for 1½ days. The percentages of cells that acquired a nonmyogenic cell fate were analyzed morphologically and immunohistochemically as described in Fig. 2. (**P* < 0.05; ***P* < 0.01.) **(B)** Effects of exogenous Wnt on muscle regeneration. Muscles of young mice were injured, and either Wnt3A (200 ng/10 µl) or control solution [10 µl of 0.1% bovine serum albumin (BSA)] was injected into regenerating tissues 1 day after injury. Cryosections were stained with Gomori stain. **(C)** Effects of Wnt inhibition on fibrosis in regenerating muscle. Muscles of young and aged mice were injured, and either DKK1 (500 ng/10 µl) or control solution (10 µl of 0.1% BSA) was injected into regenerating tissues 1 day after injury. Muscles were analyzed 5 days later. Cryosections were stained with antibodies against collagen VI (green) and embryonic myosin heavy chain (red). The histogram represents the fibrotic index (as in Fig. 1B). (***P* < 0.01; **P* < 0.05.)



tally and postnatally (17). In contrast to the inhibition of myogenesis reported here, Wnt signaling may promote myogenic lineage progression during development (18). Such pleiotropic effects may relate to differences in the timing of Wnt signaling with regard to the state of cellular differentiation or to changes in other interacting signaling pathways during development and aging. Our results may provide a strategy to improve tissue repair, particularly under conditions in which regeneration is impaired and fibrosis is favored, such as in aging and muscular dystrophies.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S9
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International Conservation Policy Delivers Benefits for Birds in Europe

Paul F. Donald,^{1*} Fiona J. Sanderson,¹ Ian J. Burfield,² Stijn M. Bierman,³ Richard D. Gregory,¹ Zoltan Waliczky¹

Conservation of the planet's biodiversity will depend on international policy intervention, yet evidence-based assessment of the success of such intervention is lacking. Poor understanding of the effectiveness of international policy instruments exposes them to criticism or abandonment and reduces opportunities to improve them. Comparative analyses of population trends provide strong evidence for a positive impact of one such instrument, the European Union's Birds Directive, and we identify positive associations between the rate of provision of certain conservation measures through the directive and the response of bird populations. The results suggest that supranational conservation policy can bring measurable conservation benefits, although future assessments will require the setting of quantitative objectives and an increase in the availability of data from monitoring schemes.

Because global threats to biodiversity are largely anthropogenic, already considerable in scale, and accelerating rapidly

(1), their solutions will depend largely on international policy intervention. This was recognized in the formulation of international agreements

such as the Convention on Biological Diversity (CBD); at least 20 regional or global conservation agreements currently exist, absorbing a high proportion of global conservation resources (2). Evaluation of the impact of international conservation policy intervention lags far behind that of most other policy fields (3), largely because of a paucity of data on the response of the species to which intervention is targeted (4, 5). This leads to a poor understanding of the cost effectiveness of the relevant policy instruments (6), reducing opportunities to improve them (7) and exposing them to criticism from both within and outside the conservation lobby (8). Although properly implemented conservation legislation can bring measurable benefits to wildlife (9–11), evaluation has hitherto had its basis either in an assessment of the provision of conservation resources, rather than the population responses of the target species to such provision, or in the responses of a small, possibly unrepresentative proportion of the species or countries to which such legislation was targeted. We assessed the impact of an international bird conservation policy that covers all member states of the European Union (EU) by using data on all the species and countries to which the agreement applies. We aimed to provide an independent assessment of the extent to which a major international policy instrument has resulted in the delivery of measurable conservation outcomes.

Biodiversity conservation legislation in the EU is founded primarily on two directives, Council Directive 79/409/EEC of 2 April 1979 on the conservation of wild birds (the Birds Directive) and Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora (the Habitats Directive). The Birds Directive set out to establish a framework and objectives for the conservation of all birds throughout the EU, although the precise legal mechanisms for achieving this aim were left to the discretion of individual member states. Central to the directive was a list (Annex I) of species considered to be particularly vulnerable or rare or to require special conservation measures (12). Member states are bound by the directive to improve the conservation status of these species by protecting or enhancing their habitats, for example, through the designation of special protection areas (SPAs) (12). Furthermore, a number of general measures to protect populations of all bird species was also agreed upon. No quantitative targets were set in the directive, so we developed five expectations that should be met for us to conclude that the directive has had a detectable positive impact. (i) We expected to

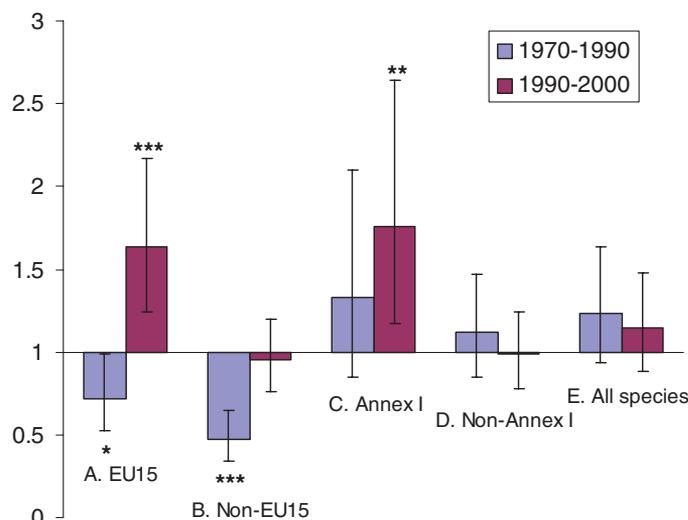
detect an improvement in the population trajectory of species listed on Annex I after the implementation of the directive, relative to that of non-Annex I species, within the original 15 member states of the EU (EU15). (ii) We expected any improvement in the population trajectory of Annex I species relative to non-Annex I species in the EU15 to be significantly greater than that recorded in parts of Europe to which the directive does not apply. (iii) We expected trends of Annex I and non-Annex I species to be more positive within the EU15 than outside it. (iv) We expected any positive impacts of Annex I listing to be most apparent in species that have been listed for longest. (v) We expected to detect a positive association across participating countries between the extent to which the directive's conservation initiatives were deployed and trends in bird populations. Our analytical approach has its basis in the statistical testing of these five expectations.

Major inventories on the status and population trends of all of Europe's breeding birds, collected at a country level and covering the periods 1970–1990 and 1990–2000 (13, 14), provided the opportunity to evaluate the impact of the Birds Directive. In each period, population trends of each species in each European country were allocated a single population trend score; these have already been published (13, 14) and formed the data we modeled to test the expectations described above. The availability of data from two time periods, and from within and outside the EU, permitted an analysis with serendipitous characteristics of a highly replicated before-after-control-impact (BACI) approach (15). This permitted comparison of trends before

and after 1990, between Annex I and non-Annex I species within the EU, and between Annex I species in the EU and the same group of species outside the EU (16). The use of a semi-experimental design based on both horizontal and longitudinal comparisons and the testing of multiple expectations maximized the likelihood that the observed patterns were causally related to variables identified by the models as having significant explanatory power.

Because the aim of the analysis was to examine the impact of Annex I listing by using trends over the period 1990–2000, we limited comparisons to data from the EU15, all but three of which joined the EU before the start of the 1990–2000 census period (17). For the same reason, we restricted our list of Annex I species to those added to the Annex before 1993 (12). Because trends were recorded in bands of unequal width (ordered from increasingly negative to increasingly positive population trends), we treated the response variable as ordinal and used the proportional odds model to assess differences between groups of species in the cumulative probabilities of being in higher ordered trend bands (16). After controlling for known variation within the database in trends between species using different habitats (18), between migrants and nonmigrants (19), and for the non-independence of trends within countries and species, we detected a highly significant effect of Annex I listing (Fig. 1). In the EU15, Annex I species were significantly more likely to be assigned to a lower population trend class than non-Annex I species in 1970–1990. However, this pattern was reversed in 1990–2000, when Annex I species were significantly more likely

Fig. 1. Odds ratios ($\pm 95\%$ confidence limits) from proportional odds models (16). Each bar indicates the ratio of two groups of species in their cumulative odds of being in a more positive trend band in one of two time periods, with significant deviations from 1 indicated ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). The odds ratios indicate how many times higher the odds were of populations of Annex I species having more positive trends than populations of non-Annex I species in the EU15 (A) and outside the EU15 (B) in each time period. Significant positive deviation from an odds ratio of 1 indicates a significantly higher probability that Annex I species had more positive trends than non-Annex I species; odds ratios that are significantly smaller than 1 indicate the reverse. The cumulative odds of species in the EU15 having a more positive population trend than those in non-EU15 countries are shown for Annex I species (C), non-Annex I species (D), and all species combined (E). All models controlled for the known effects on trend of each species' habitat and migration strategy and controlled for the non-independence of trends within species across countries.



¹Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire SG19 2DL, UK. ²BirdLife International, Wellbrook Court, Girton Road, Cambridge CB3 0NA, UK. ³Biomathematics and Statistics Scotland, University of Edinburgh, The King's Buildings, Edinburgh EH9 3JZ, UK.

*To whom correspondence should be addressed. E-mail: paul.donald@rspb.org.uk

to be recorded in a higher population trend band than non-Annex I species (Fig. 1A), thus meeting our first expectation. Outside the EU15, although trends of Annex I species improved significantly compared to those of non-Annex I species, they were no more likely to have more positive trends than non-Annex I species in 1990–2000 (Fig. 1B). The difference in trend between Annex I and non-Annex I species did not differ between the EU15 and non-EU15 countries in 1970–1990 [difference in $\log(\text{odds ratios}) = 0.17 \pm 0.22 \text{ SE}$] but was significantly greater in the EU15 than in non-EU15 countries in 1990–2000 [difference in $\log(\text{odds ratios}) = 0.52 \pm 0.18 \text{ SE}$]. Thus, our second expectation was met.

There was no significant difference in trends of Annex I species within and outside the EU15 in 1970–1990. However, by 1990–2000, Annex I species in the EU15 were significantly more likely to be recorded in a higher trend band than the same group of species outside the EU15 (Fig. 1C), a pattern that was not apparent in non-Annex I species (Fig. 1D) or across all species combined (Fig. 1E). Our third expectation was therefore partly met.

Between 1990 and 2000, species listed on Annex I of the Birds Directive fared significantly better on average than non-Annex I species within the EU15, a pattern not apparent in the same groups of species outside the EU15. This difference withstood controls for phylogenetic non-independence (16) and was due almost entirely to trends of species that had been listed on Annex I for longest (fig. S1), supporting both our fourth expectation and a previous estimate (9) that the lag between policy intervention and

a detectable population-level response exceeds 10 years. Because the effects of both habitat and migration strategy were controlled in the analyses, this difference could not be ascribed to Annex I species being disproportionately represented in habitat or migration classes that fared better than average. Nor could the difference be accounted for by the deliberate or fortuitous allocation to Annex I of species that were already increasing, because most of these species were listed well before 1990, when their trends were significantly more negative than those of non-Annex I species (Fig. 1A). Lastly, the difference in trends between Annex I and non-Annex I species in the EU could not be explained by a general global increase in Annex I species, for example in response to climate change, because outside the EU15 Annex I trends were no different from those of non-Annex I species.

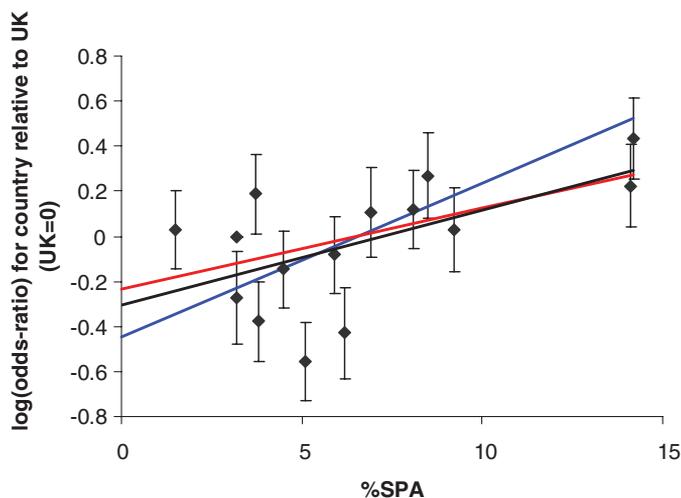
Evidence for a causal link between policy intervention and species response (our fifth expectation) was found in the positive association across EU15 countries between mean species trend and the proportion of land designated as SPAs (Fig. 2). This pattern was apparent for all species combined, and for Annex I and non-Annex I species separately, and was significantly stronger for Annex I than for non-Annex I species. Parameters of proportional odds models suggested that, for every additional 1% of a country's land area designated as SPAs, the odds of a species being in more-positive population trend classes increased by around 4% across all species and for non-Annex I species and by around 7% for Annex I species (16). The significantly stronger response of Annex I species

to the provision of SPAs is consistent with a causal link between the delivery of conservation measures through the directive and the response of the target species because SPA designation and management are targeted largely toward Annex I species.

Therefore, four of our five expectations of a positive impact of the Birds Directive were fully met, and the remaining one was partly met (populations of Annex I species, although not of non-Annex I species, had more positive trends in the EU15 than outside it). Furthermore, although trends of non-Annex I species did not differ between the EU15 and other countries, there was evidence that trends of these species were more positive within the EU15 in countries with higher deployment of SPAs. The data are therefore consistent with the hypothesis that the Birds Directive has brought demonstrable benefits to bird populations in the EU and that international policy intervention can be effective in addressing conservation issues over large geographical areas.

Our results support previous assertions (4, 5, 20) that relatively simple yet robust population monitoring can play an important role in assessing the success of supragovernmental conservation policies, as it already has in demonstrating the environmentally damaging effects of international policy in other sectors (21). Much biodiversity monitoring is undertaken by volunteers (22), making it inexpensive relative to the costs of developing and implementing international policy. If such policies were to provide support for monitoring, for example by contributing to a global monitoring network (23), their success could be evaluated. Furthermore, setting targets that are both quantitative and measurable would increase the resolution of subsequent assessments. Precise goals and specific measures for monitoring policy effectiveness should be designed and tested at the time that the policy is implemented. Otherwise, quantitative assessments of policy intervention will continue to depend on post hoc, serendipitous analyses of the type presented here. Until policy and monitoring become more integrated, the success of international conservation policies in protecting the planet's biodiversity or in achieving goals such as the CBD's 2010 target to reduce the rate of biodiversity loss (24) will be difficult or impossible to quantify. The prognosis for biodiversity is grim because this lack of feedback can only serve to weaken international policy intervention at a time of unprecedented loss.

Fig. 2. Plot of country estimates of $\log(\text{odds ratios})$ against the percentage of land area designated as SPAs in the EU15 member states for Annex I and non-Annex I species combined (means \pm SE and black regression line; slope = 0.042). The odds ratios indicate the extent to which species are more or less likely to appear in more-positive trend classes than species in the reference country (UK), which has a value of zero and no SE. In order to graphically represent this relationship, country effects of odds ratios were extracted from a proportional odds model of trend class, with migration strategy and breeding habitat also fitted as fixed effects and species fitted as a random effect. The regression coefficient differed significantly from zero ($P < 0.02$) and was estimated by fitting percentage of SPA cover directly as a covariate in a proportional odds model and including a random country effect (16). A similarly significant positive association was apparent for Annex I (regression line only shown in blue, slope = 0.068) and non-Annex I (regression line only shown in red, slope = 0.036) species separately. A significant ($P < 0.01$) interaction term indicated that the slopes for Annex I and non-Annex I species differed.



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Supporting Online Material

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Materials and Methods

SOM Text

Fig. S1

References

Appendix S1

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Adaptive Mutations in Bacteria: High Rate and Small Effects

Líliá Perfeito,¹ Lisete Fernandes,^{1,2} Catarina Mota,¹ Isabel Gordo^{1*}

Evolution by natural selection is driven by the continuous generation of adaptive mutations. We measured the genomic mutation rate that generates beneficial mutations and their effects on fitness in *Escherichia coli* under conditions in which the effect of competition between lineages carrying different beneficial mutations is minimized. We found a rate on the order of 10^{-5} per genome per generation, which is 1000 times as high as previous estimates, and a mean selective advantage of 1%. Such a high rate of adaptive evolution has implications for the evolution of antibiotic resistance and pathogenicity.

The rate at which new mutations arise in natural populations and their fitness effects are of key importance in evolutionary genetics. Classical mutation accumulation experiments have indisputably shown that among the spontaneous mutations that affect fitness, those that cause deleterious effects are far more common than those that cause increases in fitness. Whereas there are currently several direct and indirect estimates of the deleterious mutation rate in different organisms, data are lacking for beneficial mutations (1). The latter are of particular interest because they constitute the driving force of adaptation and survival of populations in new environments.

Several theoretical studies have made some general predictions about the long-term process of adaptation toward an optimum (2, 3). One prediction suggests that the effects of beneficial (advantageous) mutations (s_a) are exponentially distributed, in that many have very small effects and those that cause strong increments in fitness are rare (3). These are plausible predictions

given that organisms are in general well adapted to their environments, so only small and rare changes lead to fitness increases (4–11).

The true distribution of newly arising beneficial mutations in an organism in a given environment is difficult to estimate because the probability of fixation of a beneficial mutation that increases fitness by s_a is only $2s_a$, which means that mutations of small effect are not likely to increase in frequency. This implies that the distribution of mutations that escape stochastic loss (become fixed or reach high enough frequencies to be observed) is truncated for small values (12, 13). In addition, clonal interference occurs in large populations with a high beneficial mutation rate (U_a) and no recombination and will slow adaptation (compared to sexual populations of the same size) (14). Namely, if multiple beneficial mutations appear in different lineages, they compete with each other for fixation. This translates into an adaptation rate less than that predicted by the mutation rate and population size, and into the fixation only of mutations of large effect (15). Recently, there has been a considerable effort to predict the rate and distribution of beneficial mutations and the effect of clonal interference on the adaptation rate (16, 17).

Current estimates for U_a fall around 10^{-9} to 10^{-8} for RNA viruses and *Escherichia coli* (4, 5, 16). A similar beneficial mutation rate was estimated for *Pseudomonas fluorescens* under adaptation to stressful conditions (9). A caveat for all of these estimates is that they were obtained from populations with very large effective population size (N_e) and followed adaptation to a new environment under conditions in which clonal interference had a strong effect. This led to downward biased estimates of U_a . Here, we provide estimates for the genomic mutation rate for beneficial mutations in *E. coli* that are less biased by clonal interference.

In this work, we used populations with an intermediate effective population size—big enough that genetic drift is unlikely to drive slightly deleterious mutations to a high frequency but small enough to minimize the effects of clonal interference between beneficial mutations. To estimate the beneficial mutation rate and the distribution of fitness effects of single mutations, we used a microsatellite marker system pioneered by Imhof and Schlotterer (4). Mutations at a microsatellite locus coded by a nonconjugative plasmid can generate neutral allelic diversity in a very short time (4, 18), and selective sweeps, occurring in the bacterial genome, can be identified by following the rapid increase in the frequency of the linked microsatellite allele (4).

We allowed populations of *E. coli* to adapt to a given laboratory environment for 1000 generations and followed the allelic distribution of the microsatellite at periodic intervals. From this distribution, the number of mutations that escaped stochastic loss during this period was inferred for populations with a small effective size ($N_e = 2 \times 10^4$) and for populations with a very large effective size ($N_e = 10^7$). The latter allowed us to compare our estimates with those previously published (4, 16, 19).

The beneficial mutations that escape stochastic loss are expected to follow a gamma distribution with shape parameter 2 and with a mean

¹Instituto Gulbenkian de Ciência, Rua da Quinta Grande, number 6, 2780-156 Oeiras, Portugal. ²Escola Superior de Tecnologia da Saúde de Lisboa, Lisboa, Portugal.

*To whom correspondence should be addressed. E-mail: igordo@igc.gulbenkian.pt