

# Interspecific Patterns of Genetic Diversity in Birds: Correlations with Extinction Risk

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**Abstract:** *Birds are frequently used as indicators of ecosystem health and are the most comprehensively studied class in the animal kingdom. Nevertheless, a comprehensive, interspecific assessment of the correlates of avian genetic diversity is lacking, even though indices of genetic diversity are of considerable interest in the conservation of threatened species. We used published data on variation at microsatellite loci from 194 bird species to examine correlates of diversity, particularly with respect to conservation status and population size. We found a significant decline in mean heterozygosity with increasing extinction risk, and showed, by excluding species whose heterozygosity values were calculated with heterospecific primers, that this relationship was not dependent on ascertainment bias. Results of subsequent regression analyses suggested that smaller population sizes of threatened species were largely responsible for this relationship. Thus, bird species at risk of extinction are relatively depauperate in terms of neutral genetic diversity, which is expected to make population recovery more difficult if it reflects adaptive genetic variation. Conservation policy will need to minimize further loss of diversity if the chances of saving threatened species are to be maximized.*

**Keywords:** birds, extinction risk, genetic diversity, heterozygosity, microsatellites

Patrones Interspecíficos de Diversidad Genética en Aves: Correlaciones con el Riesgo de Extinción

**Resumen:** *Las aves son utilizadas frecuentemente como indicadores de la salud del ecosistema y son la clase del reino animal más estudiada. Sin embargo, se carece de una evaluación interspecifica, integral de las correlaciones de la diversidad genética, aun cuando los índices de diversidad genética son de interés considerable para la conservación de especies amenazadas. Utilizamos datos publicados sobre la variación en loci microsatélite de 194 especies de aves para examinar correlaciones de diversidad, particularmente con respecto al estatus de conservación y el tamaño de la población. Encontramos una declinación significativa en la heterocigosidad promedio con el incremento del riesgo de extinción, y mostramos, al excluir especies cuyos valores de heterocigosidad fueron calculados con bases heteroespecificas, que esta relación no dependió del sesgo de comprobación. Los resultados de los análisis de regresión subsecuentes sugieren que las poblaciones más pequeñas de especies amenazadas son las responsables de esta relación. Por lo tanto, las especies de aves en riesgo de extinción son relativamente pobres en términos de diversidad genética neutra, que se espera dificulte la recuperación de la población si refleja variación genética adaptativa. Las políticas de conservación deberán minimizar mayores pérdidas de diversidad para maximizar las probabilidades de salvar especies amenazadas.*

**Palabras Clave:** aves, diversidad genética, heterocigosidad, microsatélites, riesgo de extinción

## Introduction

Genetic diversity is a trait of both individuals and populations that describes the variation of alleles and genotypes

within the genome (Frankham et al. 2002). It is an important component of biodiversity (Humphries et al. 1995) and affects population characteristics such as extinction risk and evolutionary potential (Frankham et al. 2002).

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For example, Saccheri et al. (1998) report that reduced genetic diversity is associated with elevated extinction rates in a butterfly metapopulation, and circumstantial evidence suggests that genetic problems contributed to local population extinctions of the Middle Spotted Woodpecker (*Dendrocopus medius*) (Pettersson 1985) and the Heath Hen (*Tympanuchus cupido*) (Simberloff 1988). Furthermore, the phenomenon of genetic rescue, by which the immigration of new alleles into the population produces an increase in population fitness, has been reported in fish (Vrijenhoek 1994), reptiles (Madsen et al. 2004), mammals (Schwartz & Mills 2005; Stokstad 2005), and birds (Westemeier et al. 1998), providing further evidence for the importance of genetic diversity to population viability. A complementary finding is that high population-level genetic diversity provides greater evolutionary potential in the grass species *Phalaris arundinacea* (Lavergne & Molofsky 2007).

It is difficult to evaluate whether such findings represent the exception or the rule because publication bias may favor the publication of significant results. Indeed, many populations do not conform to expectations (Amos & Harwood 1998), showing little genetic variation despite maintaining a healthy population size (e.g., northern elephant seals [*Mirounga angustirostris*], Bonnell & Selander 1974 and cheetahs [*Acinonyx jubatus*], O'Brien et al. 1983). Nevertheless, with genetic diversity linked clearly to the processes of extinction and adaptation, it is evident that it should be an important character for conservationists to understand.

The World Conservation Union (IUCN) recognizes the maintenance of genetic diversity as a global conservation priority (McNeely et al. 1990), and recent analyses of measures of genetic variation (Spielman et al. 2004; Garner et al. 2005) reflect the increasing attention paid to erosion of genetic diversity by conservation biologists. It is difficult to determine whether the low levels of genetic diversity reported for a number of endangered bird species are representative because a comprehensive assessment of the class is lacking. This is surprising because the class Aves is exceptionally well studied, particularly with respect to threats of extinction, for which they are the most comprehensively studied class (Mace 1994). By carrying out a broad analysis, featuring a diverse array of bird species, we aimed to test the importance of extinction risk as a predictor of heterozygosity. This approach is an improvement on the case-by-case assessments mentioned earlier and thus should satisfy the need for empirical data on the influences of genetic diversity in natural systems (Amos & Harwood 1998).

We analyzed the relationship between microsatellite marker diversity and a number of potential factors that contribute to variation in genetic diversity, including life-history variables and extinction risk collated from the literature. We used both raw species values and independent contrasts that accounted for phylogenetic rela-

tionships among species. From these, we identified extinction risk as a highly significant predictor of heterozygosity, which remained in the model even after removal of studies in which ascertainment bias could have acted. The results of our incorporation of an index of population size suggest that the low genetic diversity of threatened species is likely to be a result of small population size.

## Methods

### Measures of Genetic Diversity

Variable microsatellite loci, such as those used by molecular ecologists, have a particularly high degree of polymorphism, with heterozygosity frequently exceeding 70% (Webster et al. 2002). We used microsatellite diversity as a surrogate for overall genetic diversity for the following reasons. First, it has been a popular choice of marker for assessing genetic diversity in wild populations for the past decade. Second, markers isolated in one species can often be applied to other species. These 2 aspects ensure that there is a large sample size in terms of the number of species available for analysis. Third, although there are weak associations between microsatellite diversity and fitness within species (e.g., Coltman & Slate 2003), the majority of allelic variation at microsatellite loci is likely to be neutral. Last, levels of variation in populations are often considered representative of adaptive genetic diversity (Garner et al. 2005). Although there is some controversy surrounding how close the relationship is between neutral and adaptive diversity across species (e.g., Merilä & Crnokrak 2001; Reed & Frankham 2001), there is at least evidence of a weak positive relationship (Reed & Frankham 2001).

Two measures may be calculated from microsatellite data and used as indicators of genetic variation in a population: heterozygosity and allelic richness. Heterozygosity reflects the proportion of heterozygotes within a population, whereas allelic richness represents the number of alleles at each locus. The latter depends more heavily on sample size (Leberg 2002) and the range over which samples were collected (Garner et al. 2005), so we used heterozygosity values for all analyses. Microsatellite data were sourced from the peer-reviewed literature to obtain a quantitative description of genetic diversity among bird populations. First, we reviewed 3 journals (*Conservation Genetics*, *Molecular Ecology*, and *Molecular Ecology Notes*). We examined all issues up to December 2005. Second, we searched Web of Knowledge (<http://wok.mimas.ac.uk>) by entering search criteria such as "bird AND microsatellite." Third, we used the Sheffield Molecular Genetics facility's list of bird microsatellite loci (<http://www.shef.ac.uk/misc/groups/molecol/smgf.html>) as a cross-reference to identify sources of heterozygosity data that we

had missed. Data on domesticated species or captive populations were excluded. Where both observed and expected heterozygosity data were available, we used expected heterozygosity because it is less affected by sample size (Nei 1978) and the occurrence of null alleles (Callen et al. 1993). Where multiple studies had been carried out on a species, we chose a single study on the basis of maximizing the number of loci analyzed and the sample size (increasing these improves the accuracy of a heterozygosity score). Overall, data from 194 species were included (see Supplementary Material).

### Predictor Variables

We examined the following predictor variables: extinction risk, body mass, clutch size, and sample size. We derived extinction risk from the 2006 IUCN Red List (IUCN 2006), which is available as a Web-based database (<http://www.redlist.org>) and provides a comprehensive list of bird species and their extinction risk classification. Using this list, we assigned each species an integer score on a numerical index from 1 to 5 (1, least risk category; 2, near threatened; 3, vulnerable; 4, endangered; 5, critically endangered), as has been done previously to assess predictors of extinction risk (e.g., Bennett & Owens 1997; Purvis et al. 2000b; Cardillo et al. 2005). Following Purvis et al. (2000b), the resulting measure of extinction risk was treated as a continuous character, which is justifiable if the red-list categories are considered discrete approximations to an underlying continuum. Of the 194 species, 147 (75.8%) were of least concern, 10 (5.2%) were near threatened, 21 (10.8%) were vulnerable, 13 (6.7%) were endangered, and 3 (1.5%) were critically endangered.

For the body mass, we used the mean of male and female mass derived from data in Dunning (1992). When data were not available for a species ( $n = 14$  cases), we used the mean of all listed congeneric species.

Clutch size is used as an index of general life-history strategy because it captures general life-history variation well (Bennett & Owens 2002) and is available for most species. Modal clutch size was obtained from Hoyo et al. (1992). Where a range of clutch sizes was given, the value halfway between the extremes was used, following Stuart-Fox and Owens (2003). Where species data were not available, we used the family-level average reported by Bennett and Owens (2002).

In accordance with the central-limit theorem, increased sample size improves the accuracy of any measurement. Where the sample size varied between loci, we used the mean sample size. It seemed possible that threatened species might tend to have smaller sample sizes as a result of smaller population size or lower population density; therefore, we considered it worthwhile to test for a relationship between sample size and heterozygosity.

### Phylogeny

We constructed a composite species-level phylogeny on the basis of data from molecular phylogenetic studies (see Supplementary Material). Sibley and Ahlquist's (1990) phylogeny provided lower-order relationships, which were, for some families, sufficiently detailed to allow all intrafamilial relationships to be elucidated. Nevertheless, for many families we used additional phylogenetic information, as follows: Phasianidae (Randi 1996; Dimcheff et al. 2002); Anatidae (Livezey 1995; Donnell-Goussé et al. 2002); Cuculidae (Aragón et al. 1999); Psittacidae (de Kloet & de Kloet 2005); Laridae (Friesen et al. 1996; Chu 1998); Falconidae (Cade 1982; Nittinger et al. 2005); Accipitridae (Lerner & Mindell 2005); Spheniscidae (Giannini & Bertelli 2004); Procellariidae (Kennedy & Page 2002); Corvidae (Barker et al. 2004; Ericson et al. 2005); Paridae (Gill et al. 2005); Sylviidae (Helbig & Seibold 1999; Cibois 2003; Drovetski et al. 2004); Fringillidae (Lovette & Bermingham 1999; Lovette & Bermingham 2002; Yuri & Mindell 2002; Zink 2002; James 2004). Where uncertainty remained as to the order of bifurcation, we used a polytomy.

### Comparative Analyses

We  $\log_{10}$  transformed all predictor variables so that their distributions were approximately normal. Because all species in a monophyletic grouping share a common ancestor, they cannot be considered as independent data points in statistical analyses (Garland et al. 1992). Phylogenetic nonindependence is a likely phenomenon in conservation-related data (Fisher & Owens 2004). There is ample evidence that extinction risk follows a phylogenetically nonrandom distribution across avian families (Bennett & Owens 1997; McKinney 1997; Purvis et al. 2000a), as in other taxa (Cardillo & Bromham 2001; Duncan & Lockwood 2001; Jones et al. 2003). Likewise, ecological and morphological features tend to be influenced by phylogeny (Harvey & Pagel 1991; Bennett & Owens 2002). To account for such potential nonindependence in our data set, we used our phylogeny to generate phylogenetically independent contrasts with the CRUNCH algorithm of the Comparative Analysis by Independent Contrasts (CAIC) computer package (Purvis & Rambaut 1995). In the absence of complete branch-length information, we assumed branches were of equal length. Following the suggestion of Price (1997), we report the results of analyses of both raw species values and on phylogenetically independent contrasts. We use the term *species correlation* to refer to the correlation between the mean values for species and *contrast correlation* to describe the correlation between the independent contrasts obtained by transforming the species values.

Pairwise correlation coefficients were calculated with SPSS (version 14.0: SPSS, Chicago, Illinois). We used a

multiple stepwise regression model to test for associations between the predictor variables and heterozygosity. To perform this, we used SPSS to follow a backward model-selection process, which produced a minimum adequate model (MAM;  $p = 0.1$  for retention). All regressions with phylogenetically independent contrasts were forced through the origin (Garland et al. 1992).

Following the initial analyses on all 194 species, we repeated the approach on a restricted subset of species, which excluded those for which heterospecific primers (those originally cloned in another species) had been used, which yielded a data set of 103 species. This eliminated the possibility of a confounding influence caused by ascertainment bias, which occurs when there are differences in the properties of microsatellites amplified in their original species and in another species (Ellegren et al. 1995; Primmer et al. 1996). In general, lower microsatellite diversity is reported in studies in which heterospecific primers were compared with conspecific primers.

A second subset of species was selected from the overall data set on the basis of availability of population size estimates, with data taken from the *Birds of the World* series (Hoyo et al. 1992). When estimated population size was given as a range, we used the midpoint value. Values were  $\log_{10}$  transformed to provide an approximately normal distribution. This data set, containing 89 species, was used to build a model describing variation in heterozygosity as before, with the difference that population size was included as a sixth predictor variable. We expected that population size would correlate strongly with extinction risk, so it was not included in the initial model. Our aim here was to determine the extent to which adding population size to the models accounted for any relationship between genetic diversity and extinction risk.

For those genera in the data set represented by multiple species of differing extinction risk classification ( $n = 15$ ; see Supplementary Material), we carried out a pairwise analysis to determine whether there was a within-genus trend relating heterozygosity and extinction risk. Within each of the 15 genera, species were selected so as to maximize the difference in the extinction-risk score. If more than one species shared the same extinction score, a random selection was made. We used a paired  $t$  test to determine whether there was a significant change in mean heterozygosity level, across species and within-genera, as extinction risk increased.

## Results

We located data for 194 species that could be described by all 5 variables: heterozygosity and 4 predictor variables (extinction risk, sample size, body mass, and clutch size). Associations between the predictor variables were

**Table 1.** Assessment of associations between variables entered into models of heterozygosity, with Pearson product moment correlation coefficient given.<sup>a</sup>

	Extinction risk	Sample size	Body mass	Clutch size
Extinction risk		−0.067	0.204 <sup>b</sup>	−0.103
Sample size	−0.065		−0.109	0.009
Body mass	−0.035	0.062		−0.050
Clutch size	0.055	−0.006	−0.117	

<sup>a</sup>Figures above the diagonal are for species correlation, whereas figures below the diagonal represent contrast correlation;  $n = 194$  and 183 for raw species correlations and contrast correlations, respectively.

<sup>b</sup> $p < 0.01$ .

limited (Table 1). Nevertheless, we used a multivariate model of heterozygosity.

Two variables explained a significant portion of the variance in heterozygosity for species correlation: extinction risk and body mass ( $F_{2,191} = 31.5$ ,  $p < 0.0005$ ). The minimal adequate model (MAM) explained 24% ( $r^2_{\text{adj.}} = 0.240$ ) of the total variation in heterozygosity. The slopes of these variables indicated that both had a negative relationship with heterozygosity (Table 2; Fig. 1). The MAM generated for contrast correlation by backward stepwise multiple regression featured a single variable: extinction risk ( $F_{1,182} = 37.3$ ,  $p < 0.0005$ ). The model explained 17% ( $r^2_{\text{adj.}} = 0.165$ ) of the variation in heterozygosity, with the slope indicating a negative relationship with heterozygosity (Table 2), which agreed with results of the previous model. Because the multiple regression analysis was forced through the origin in this case, the  $r^2$  value could not be compared with that of the previous MAM, which included an intercept. When the analysis was repeated, with critically endangered species excluded, the MAMs for both species correlation and contrast correlation were unaltered.

After excluding those species for which heterozygosity values had been estimated with heterospecific primers, 103 species remained. Backward stepwise multiple regression produced a MAM for species correlations containing 3 predictor variables: extinction risk, sample size, and body mass ( $F_{3,99} = 13.3$ ,  $p < 0.0005$ ). This model explained 27% ( $r^2_{\text{adj.}} = 0.265$ ) of the variation in heterozygosity. The slopes of the parameter estimates

**Table 2.** Results of the multiple-regression model that tested for associations between predictor variables and mean heterozygosity.

Analysis	Variables	Coefficient	SE	t	p
Species correlation	variables included in MAM*				
	extinction risk	−0.061	0.010	−5.92	<0.0005
	body mass	−0.046	0.012	−3.98	<0.0005
	variables excluded from final model				
	sample size	−0.011	0.021	−0.535	0.594
Contrast correlation	clutch size	0.042	0.045	0.945	0.346
	variables included in MAM				
	extinction risk	−0.063	0.010	−6.11	<0.0005
	variables excluded from final model				
	sample size	−0.007	0.022	−0.338	0.736
	clutch size	0.034	0.081	0.423	0.673
	body mass	−0.027	0.028	−0.954	0.341

\*Minimum adequate model.

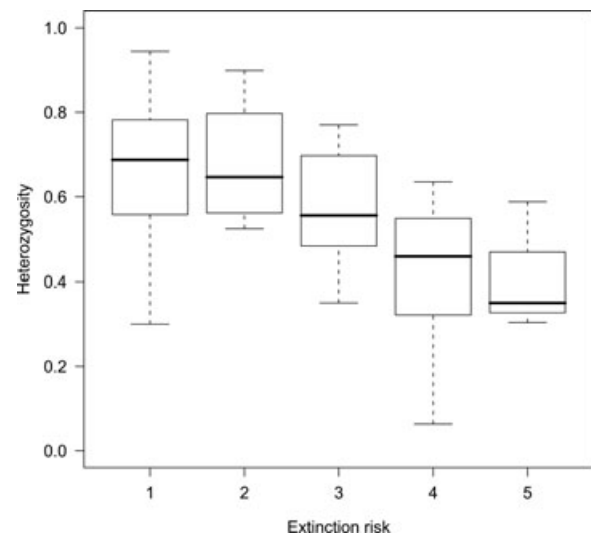
indicated that the 3 variables had a negative relationship with heterozygosity (Table 3). For the contrast correlations, the MAM consisted of extinction risk and sample size ( $F_{2,99} = 12.9$ ,  $p < 0.0005$ ), both of which had a negative relationship with heterozygosity (Table 3). The model explained 19% ( $r^2 = 0.191$ ) of the variation in heterozygosity.

Analyzing only those species for which estimates of population size were available yielded a further restricted data set of 89 species. Backward stepwise multiple regression was carried out for this data set with 5 predictor variables (extinction risk, body mass, clutch size, sample size, and population size). For species correlations MAM consisted of 2 variables, population size and sample size ( $F_{2,86} = 15.1$ ,  $p < 0.0005$ ), that explained 24% ( $r^2_{\text{adj.}} = 0.242$ ) of the variation in heterozygosity. The slopes of these variables indicated a positive relationship between population size and heterozygosity (see Fig. 2), but a negative relationship between sample size and heterozygosity (Table 4). The contrast correlation model from the same 89 species featured population size and sample size ( $F_{2,81} = 7.87$ ,  $p = 0.001$ ) and explained 14% ( $r^2 = 0.142$ ) of the variance in heterozygosity. The slopes of these variables showed that population size had a positive relationship and sample size had a negative relationship with heterozygosity (Table 4). Hence, in both of these analyses, inclusion of estimates of population size resulted in this variable being retained in the model in preference to extinction risk. As expected, population size and extinction risk were highly and significantly correlated, regardless of whether raw species values or independent contrasts were considered ( $r_{89} = -0.548$ ,  $p < 0.0005$ ;  $r_{83} = -0.545$ ,  $p < 0.0005$ , respectively).

When we employed a matched-pairs approach, we compared average heterozygosities in 15 pairs of species and found that in 80% of cases, heterozygosity was lower in the more threatened species. Heterozygosity was on average 25% lower in species at greater risk of extinction, a significant difference (paired  $t_{14} = -2.42$ ;  $p = 0.030$  [2 tailed]).

## Discussion

Variation in genetic diversity across the class Aves has received surprisingly little attention. In our analysis of variability at microsatellite loci across species, we found a small number of robust predictors of diversity at the species level: species at greater risk of extinction had lower mean heterozygosities. This relationship remained significant when the most threatened class of species was removed, suggesting that the decline in genetic diversity was present well before the species reached a critical stage. For a subset of species, for which estimates of population size were available, extinction risk was replaced



**Figure 1.** The relationship between mean heterozygosity and IUCN extinction risk for 194 species of birds. Each boxplot depicts the median boxed within the first and third quartiles, with the dashed lines showing 1.5 times the interquartile range. Extinction-risk scores are produced by converting the red-list threat categories into integer values, as detailed in the methods.

**Table 3.** Results of the multiple-regression model that tested for associations between predictor variables and heterozygosity after excluding from the data set species for which heterozygosity values were calculated with heterospecific microsatellite primers.

Analysis	Variables	Coefficient	SE	t	p
Species correlation	variables included in MAM*				
	extinction risk	−0.068	0.015	−4.66	<0.0005
	body mass	−0.036	0.014	−2.52	0.013
	sample size	−0.062	0.027	−2.33	0.022
	variables excluded from final model				
	clutch size	0.085	0.059	1.46	0.148
Contrast correlation	variables included in MAM				
	extinction risk	−0.071	0.015	−4.61	<0.0005
	sample size	−0.055	0.028	−1.99	0.049
	variables excluded from final model				
	clutch size	0.009	0.081	0.114	0.909
	body mass	−0.033	0.030	−1.11	0.269

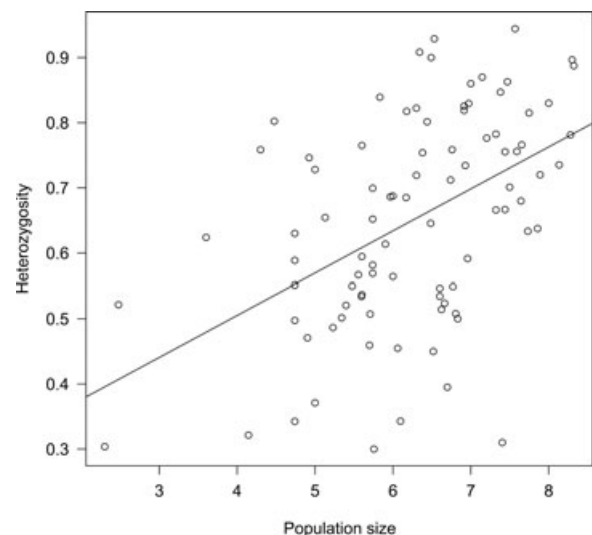
\*Minimum adequate model.

by population size as the best predictor of extinction risk. To the best of our knowledge, comparative analyses with respect to microsatellite diversity in birds are limited to 2 studies (Primmer et al. 1996; Petrie et al. 1998), neither of which tested for a correlation with extinction risk. The 194 species in our data set compares favorably with the sample sizes used by Garner et al. (2005), who used 108 mammal species in their assessment of patterns of genetic diversity, and Spielman et al. (2004), who paired 170 threatened plant and animal species with nonthreatened relatives to assess whether genetic factors affect threatened species. Spielman et al. (2004) report that heterozygosity is 35% lower in threatened species, which compares well with the results of our matched-pairs analyses, where heterozygosity was 25% lower in species at greater risk than in congeners at lower risk of extinction. Similarly, in our data set of 194 species, mean heterozygosity was 39% lower in species at greatest risk of extinction (endangered and critically endangered categories combined) than species of minimal conservation concern (least concern). Given the negative relationship between heterozygosity and extinction risk we found, and that has been reported recently by other workers (Spielman et al. 2004; Garner et al. 2005), this seems a robust result.

That extinction risk was a highly significant predictor of heterozygosity, with a moderate effect size, is noteworthy given the sampling error and lack of resolution in the variables we used. The categories used in the IUCN's Red List, although hierarchical, are partly subjective, and their discontinuity implies loss of potentially useful information (not all species in a category are equivalent). Similarly, microsatellite heterozygosity measures are probably subject to considerable sampling error: studies employ varying methodologies, with a diversity of research aims, and score a relatively small proportion of neutral variation. In this light the variation in microsatellite heterozygosity explained by our models is respectable. Yet, the correlation between heterozygosity and extinction

risk does not imply causation; inclusion of a measure of population size resulted in exclusion of extinction risk from the models, suggesting a potential causative agent for the relationship. Although theoretical (Tanaka 1998) and empirical (Fagan & Holmes 2006) evidence is accumulating in favor of the operation of Gilpin and Soule's (1986) "extinction vortex" in dwindling populations, it is more likely that other factors bring about the initial reduction in population size. It is also important to stress that relationships reported here do not imply that assessing genetic diversity would provide a means to assess conservation priorities; clearly, a host of other factors is involved in determining the viability of individual species.

Reduced genetic diversity indicates that population fitness is compromised (Spielman et al. 2004), which is likely to increase the threat of extinction further and



**Figure 2.** The relationship between mean heterozygosity and estimated population size for 89 species of birds. The line shows the least-squares regression. Population size is on a  $\log_{10}$  scale.

**Table 4.** Results of the multiple-regression model that tested for associations between predictor variables and heterozygosity.<sup>a</sup>

<i>Analysis</i>	<i>Variables</i>	<i>Coefficient</i>	<i>SE</i>	<i>t</i>	<i>p</i>
Species correlation	variables included in MAM <sup>b</sup>				
	population size	0.071	0.013	5.48	<0.0005
	sample size	−0.052	0.029	−1.82	0.072
	variables excluded from final model				
	clutch size	−0.008	0.055	−0.136	0.892
	extinction risk	−0.017	0.025	−0.671	0.504
Contrast correlation	body mass	−0.027	0.021	−1.30	0.197
	variables included in MAM				
	population size	0.057	0.015	3.80	<0.0005
	sample size	−0.049	0.027	−1.78	0.079
	variables excluded from final model				
	extinction risk	−0.007	0.027	−0.260	0.796
	body mass	0.014	0.035	0.411	0.682
	clutch size	0.130	0.119	1.09	0.278

<sup>a</sup>Only those species for which population-size data were available were included.

<sup>b</sup>Minimum adequate model.

perhaps lead the species into terminal decline via the extinction vortex. A logical next step would be to analyze population-level patterns of genetic diversity in bird species. By selecting appropriate species, in which there are populations characterized by different demographic trends, the possibility that the loss of genetic diversity is having a negative impact on fitness could be examined. Such an approach showed that low genetic diversity affects fitness in large felids (Packer et al. 1991; Roelke et al. 1993).

It is possible that a negative relationship between heterozygosity and extinction risk might result from a publication bias (Amos & Harwood 1998) if investigators expect, a priori, to find low genetic diversity, particularly if researchers examining healthy populations initially fail to find highly variable markers and continue their search through the genome until they find them. Nevertheless, we believe such a bias is unlikely to be operating here because workers isolating and characterizing novel microsatellite primers in all species, including those at risk of extinction, focused on finding and reporting polymorphic markers. Monomorphic loci are of little use.

It is difficult to reconcile the reduced heterozygosity of bird species at risk of extinction with Amos and Balmford's (2001) assertion that, "the loss of heterozygosity is extremely slow compared with the timescales over which conservation biology operates." The importance of our findings to conservation biology is highlighted by the fact that heterozygosity is considered less sensitive to population bottlenecks than other measures of genetic diversity, such as allelic diversity (Amos & Balmford 2001), which has been suggested by some authors (Allendorf 1986; Fuerst & Maruyama 1986) as more important than heterozygosity for evolutionary potential. If so, the effect on threatened species may be even greater. Together with the results of Garner et al. (2005), our results suggest that isolated examples of increased mean heterozygosity

at the population level following population collapses (Bancroft et al. 1995; Pemberton et al. 1996) are unrepresentative of the overall trend in vertebrates.

The correlation between extinction risk and heterozygosity, found for both raw species values and phylogenetically independent contrasts, should be of great interest to conservationists. Nevertheless, the strong correlation between extinction risk and heterozygosity does not indicate causality. A negative relationship such as this could result from ascertainment bias (Ellegren et al. 1995) if markers are less likely to be isolated and characterized in endangered species and workers instead rely on heterospecific amplification. This effect is reported by Garner et al. (2005) in their analysis of heterozygosity and extinction risk in mammals. After removing from our data set those species for which heterospecific primers were used, extinction risk remained in both MAMs, although the model's predictive power was reduced (Tables 1 & 2). Hence, although our main conclusions are not altered by eliminating the effects of ascertainment bias, it does affect heterozygosity assessments in bird populations and should continue to be a consideration in comparative studies.

That the paired MAMs differed suggests phylogeny has significant effects. We included weight in the first 2 MAMs of species correlations as a predictor of heterozygosity, yet its absence from contrast-correlation MAMs demonstrated that its inclusion in species correlations was an artifact resulting from phylogenetic effects. Clutch size was excluded from all models; therefore, we conclude that life-history variation contributed little to patterns of heterozygosity in birds. Sample size was included in both MAMs for which ascertainment bias had been eliminated, showing that it was a predictor of heterozygosity. Nevertheless, because sample size is not an inherited character and is highly unlikely to be correlated with one, interpreting its presence in the MAM

for contrast correlations is potentially misleading. Nevertheless, its inclusion in the MAM for species correlations, with a negative correlation coefficient, suggests that studies with small sample sizes report exaggerated heterozygosities.

When we added a measure of population size to these analyses, extinction risk was dropped from the MAM, which suggests that much of the variation in mean heterozygosity can be attributed to differences in population size (Neff & Gross 2001). That population size was a more powerful predictor of heterozygosity than extinction risk should not be surprising. Of the 89 species entered into the model, 80 were classified as least concern, and with so many species having the same score for this variable, its statistical power was much lower than that of population size. Among birds, insular species with restricted ranges are especially vulnerable to extinction (Hughes 2004), and our results suggest that in such species genetic factors may be involved: both insularity and low range size are likely to be associated with small breeding populations. With genetic diversity being linked to population persistence and population size, it seems reasonable to suppose that an extinction vortex could take effect, with demographic and genetic effects interacting in small populations to increase the probability of extinction (Westermeier et al. 1998).

We conclude that low genetic diversity is associated with high risk of extinction, even after accounting for ascertainment bias and several potential confounding variables. This relationship appears to result from the lower population sizes of threatened species. As typified by the IUCN Red List, much conservation effort considers the species as the key unit of concern (Garner et al. 2005), yet many studies we examined in which microsatellite data were used analyzed the diversity of a single population. Extinction rates of populations are estimated to be 3 orders of magnitude greater than species (Hughes et al. 1997), so considerable loss of genetic diversity is likely to occur before conservation action is considered. By the time a species becomes of conservation concern, it may already have lost a large fraction of its genetic diversity (Garner et al. 2005) and be entering the extinction vortex. We therefore suggest that future conservation policy ensures that effort is devoted to maximizing genetic diversity of the species genome, which may not be the case in current species-based conservation approaches.

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## Supplementary Material

A summary of the data set (Appendix S1) is available as part of the on-line article from <http://www.blackwell-synergy.com/>. The author is responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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