The successful founder: genetics of introduced *Carduelis chloris* (greenfinch) populations in New Zealand

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The amount and distribution of genetic variation in seven introduced New Zealand populations of *Carduelis chloris* were assessed at 40 loci using starch gel electrophoresis and compared with those in native European populations. Fewer alleles (1.45) and fewer polymorphic loci (33 per cent) were detected across the introduced populations than across native populations (1.75, 55 per cent), reflecting the narrow geographical origin of the introduced populations. There was no evidence for severe inbreeding or genetic drift as the levels of average heterozygosity ($H = 0.025$), percentage polymorphic loci ($P = 16.9$) and average number of alleles per locus ($A = 1.22$) were indistinguishable from levels observed within European populations ($H = 0.025$, $P = 14.1$, $A = 1.19$). Furthermore, introduced populations were genetically less weakly differentiated ($F_{ST} = 0.022$) than native populations ($F_{ST} = 0.041$), indicating that little genetic drift has been involved in the colonization of the new range. Similar levels of genetic variability in native and introduced greenfinch populations are consistent with theoretical expectations as the founder population size was relatively large (> 60 individuals) and a rapid increase in population size directly after colonization was documented. A review of earlier studies on introduced birds also revealed that reductions in levels of genetic variability seem to be inversely proportional to the size of the founder population, and that less variation has been lost if the rate of population growth directly after introduction was fast.

Keywords: bottleneck, *Carduelis chloris*, heterozygosity, introduced species.

Introduction

Colonization events, including human-assisted introductions, often entail severe reduction in population size and permanent isolation from a larger parental population. The genetic consequences of the founding event may include rapid alteration and loss of genetic variation (Nei *et al.*, 1975; Templeton, 1980; Watterson, 1984) and sometimes changes of sufficient magnitude to influence the evolutionary fate of the species (Barton & Charlesworth, 1984; Carson & Templeton, 1984). Both theoretical (e.g. Nei *et al.*, 1975) and experimental (Verspoor, 1988; Polans & Allard, 1989; McCommas & Bryant, 1990; Leberg,

1992) results emphasize the importance of the propagule size and the rate of population growth. The reduction in genetic variability arising from sampling errors (some allelic forms not included in the propagule, some lost by chance) and inbreeding is inversely proportional to the initial size of the founder population and the length of time it remains small (Nei *et al.*, 1975).

Although population bottlenecks have been rather frequently invoked to explain low genetic variability in natural populations, there are few studies where both the actual magnitude of the bottleneck and its genetic consequences have been established (but see: Bonnell & Selander, 1974; Hoelzel *et al.*, 1995). General exceptions, however, are the many human-assisted introductions where the number of founding individuals is often known quite accurately (e.g. Thomson, 1922; Long, 1981). Several studies of

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introduced plant (e.g. Schwaegerle & Schaal, 1979; Clegg & Brown, 1983; Glover & Barret, 1987) and animal (e.g. Taylor & Gorman, 1975; Baker & Moored, 1987) populations have found reduced levels of genetic variability in introduced as compared to native populations, although equally many have failed to confirm this (e.g. plants: Burdon & Brown, 1986; Warwick *et al.*, 1987; Molina-Freaner & Jain, 1992; Novak & Mack, 1993; animals: Ross, 1983; Baker, 1992). There are several possible explanations for these discrepancies which can be classified into two categories: technical and biological. Technical problems which could account for the failure to detect a change in genetic parameters, even though it has in fact happened, include generally invariable allozyme markers, small and variable sample sizes and, hence, inconclusive statistical tests. Biological explanations for lack of reduction in genetic variability following a bottleneck include maintenance of large effective population size during the bottleneck and fast population growth directly after the passage through the bottleneck.

The aim of this study was two-fold. First, to estimate levels of genetic variation within and among seven introduced populations of greenfinches in New Zealand, and to compare them with corresponding values from the native populations in Europe. Secondly, to review the earlier surveys of genetic variability of introduced bird species and relate the changes in genetic parameters with the known circumstances around their introduction.

Methods

The study species and introduction history

The greenfinch (*Carduelis chloris*) is a medium-sized seed-eating passerine bird native to the western Palaearctic region. It is a gregarious, partially migratory species which inhabits forest edges, grasslands with scattered bushes and cultivated areas such as parks and gardens (Cramp & Perrins, 1994). Breeding takes place in loose colonies, and two to three clutches are laid annually (Cramp & Perrins, 1994). As for many other birds, the greenfinch was successfully introduced to New Zealand and Australia during the 19th century. What is known about the introduction history in New Zealand is that several releases took place between 1862 and 1868. In 1862, the Nelson Acclimatisation Society introduced five birds, but there are no further records of them (Long, 1981). A pair of birds which produced at least two successful clutches in the wild was released

in 1863 at Christchurch (Lever, 1987). The Auckland Society released several birds in 1865, 18 in 1867, and 33 in 1868 (Long, 1981; Lever, 1987) and the Otago Society released eight birds in 1868 (Long, 1981). Hence, at least 64 individuals were recorded as being released, but the true number might have been higher. By 1870 greenfinches were considered to be 'so well established that no further releases were necessary' (Lever, 1987) and by 1920 they were reported to be abundant in all the settled parts of the country (Thompson, 1922), and later dispersal to several offshore islands testifies to the success of colonization (e.g. Lever, 1987).

General methods

The material for this study was collected in November 1992 and 1993 from seven localities in the two main islands of New Zealand (Fig. 1). Sample sizes with associated collecting dates are given in Table 1. Pectoral muscle, heart, liver and kidneys were removed from birds in the field, frozen in liquid nitrogen, and maintained at -80°C in the laboratory. Homogenates of tissue mixture were surveyed electrophoretically for the products of 40 enzyme loci using horizontal starch gel electrophoresis. The loci and methods were the same as used in an earlier survey of the genetic variability of native greenfinch populations (Merilä *et al.*, 1996). In short, the 40 scored loci and their Enzyme Commission number (International Union of Biochemistry and Molecular Biology, 1992) were: *ACON 1* and 2 (4.2.1.3), *ACP* (3.1.3.2), *AK 1* and 2 (2.7.4.3), α -*GPDH 1* and 2 (1.1.1.8), *CK 1*, 2 and 3 (2.7.3.2), *EAP 1* and 2 (3.4.23.24), *ES 1*, 2 and 3 (3.1.1.1), *FL-ES* (3.1.1.1), *G6PDH* (1.1.1.49), *GLUD* (1.4.1.2), *GOT 1* and 2 (2.6.1.1), *GP 1*, 2 and 3 (non-specific protein, 'general protein'), *GPI* (5.3.1.9), *ICD 1* and 2 (1.1.1.42), *LDH 1* and 2 (1.1.1.27), *MDH 1* and 2 (1.1.1.37), *MPI* (5.3.1.8), *NP* (2.4.2.1), *PEP 'A'* and *'B'* (3.4.11), *6PGD* (1.1.1.43), *PGM 1* and 2 (5.4.2.2), *SOD 1* and 2 (1.15.1.1) and *SDH* (1.1.1.14). Details of electrophoretic conditions can be found in Merilä *et al.* (1996).

Statistical analyses

Allele frequencies were calculated from genotype frequencies observed in zymograms using the BIOSYS-1 computer program (Swofford & Selander, 1989). Genotype frequencies were tested for Hardy-Weinberg equilibrium with exact tests using the GENEPOP package (Raymond & Rousset, 1995a,b). Adjustment for multiple tests was made by using the

sequential Bonferroni correction (Rice, 1989) as suggested by Lessios (1992). All loci were also tested for linkage disequilibrium using exact tests (Raymond & Rousset, 1995a); significant deviations from linkage equilibrium were detected in four of the 69 possible tests ($P < 0.05$). However, these cases involved different pairs of loci and were not significant after sequential Bonferroni correction. For

Fig. 1 Map of the collecting localities of introduced populations of greenfinch, *Carduelis chloris*, populations in New Zealand.

each population, we computed the average observed (direct count) and average expected heterozygosity (unbiased estimate of Nei, 1978) using BIOSYS-1. Differences among populations in average heterozygosity and mean number of alleles per locus were assessed using the Kruskal–Wallis analysis of variance (Sokal & Rohlf, 1981; Archie, 1985).

Recent population bottlenecks are predicted to produce an excess of alleles segregating in low frequencies (Chakraborty *et al.*, 1980; see also Barrowclough *et al.*, 1985). Therefore, we compared observed and expected numbers of rare ($q \leq 0.05$) and common alleles ($p > 0.95$) in each of the study populations. The expected number of alleles in these frequency classes was calculated using the infinite-allele model with varying mutation rate (Model IV; Chakraborty *et al.*, 1980). The discrepancy (R^2) between expected (E) and observed (O) number of alleles in a given frequency class was calculated as: $R^2 = (O - E)^2 / E$, and its significance was tested using a randomization procedure with 9999 permutations.

F -statistics were used to investigate the extent of population structuring and genetic differentiation. F_{ST} , defined as a standardized genetic variance among (sub)populations, was estimated using two different algorithms. First, to allow comparison with other studies, we calculated G_{ST} using the BIOSYS-1 (Swofford & Selander, 1989) program to obtain Nei's (1986) estimate of F_{ST} . However, several lines of evidence indicate that Weir and Cockerham's (1984) theta (θ) estimator is a more accurate descriptor of F_{ST} as it includes correction terms for sample size and the number of populations examined (Weir & Cockerham, 1984; Porter, 1990; Wolf & Soltis, 1992; Cockerham & Weir, 1993). Hence, we also calculated θ estimates using the FSTAT program where significance testing of θ was carried out by jackknifing over loci (Goudet, 1995). Pairwise G_{ST} and θ estimates were calculated using Slatkin's (1993) isolation-by-distance program.

Table 1 Greenfinch collecting localities, their coordinates, collecting dates and sample sizes (n)

Locality	Acronym	Island	Coordinates	Collecting date	n
Auckland	AUC	North	36°40' 174°28'	November 1993	42
Taupo	TAU	North	38°42' 176°06'	November 1993	15
Napier	NAP	North	39°29' 176°58'	November 1993	42
Wellington	WEL	North	40°11' 175°23'	November 1992	29
Hope	HOP	South	41°21' 173°09'	November 1993	40
Eyrewell	EYE	South	43°18' 172°11'	November 1993	39
Karitane	KAR	South	45°52' 170°30'	November 1993	39
				Σ	246

Multilocus genetical comparisons among samples were made using Rogers's (1972) genetic distances. Unlike F_{ST} , genetic distances are not affected by levels of polymorphism within populations, and therefore they provide a way of assessing differentiation on an absolute scale. Rogers's distances were subjected to cluster analysis by using the Neighbour-Joining method of Saitou & Nei (1987) as implemented in PHYLIP (Felsenstein, 1993). Fifteen different random orderings of populations were tried to ensure that the resulting trees were not sensitive to the input order of populations.

To examine the relationship between geographical distance and different measures of genetic differentiation, a matrix of geographical distances between sites was calculated using the geographical coordinates for each site (Table 1). To avoid the distortion affecting ordinary map projections, this was carried out by measuring the shortest distance between two points as given by the great circle track connecting them (e.g. Korsström, 1927). Statistical testing of all matrix correlations (r_M) was carried out using normalized Mantel statistics (Z) with a randomization approach (e.g. Manly, 1991). Five thousand random permutations were used and P taken as the proportion of permutations with Z -values higher than the observed.

Comparative analyses

The data used in the comparative analyses include six earlier bird studies where information on genetic variability was available both from native and introduced ranges. Where variability estimates were not given in the respective publications, they were estimated from allele frequency data using BIOSYS-1. Information on history of introduction was obtained from the publications and from Thomson (1922), Long (1981) and Lever (1987). The total numbers of individuals recorded in these references were taken as the sizes of the founder populations. For most species, this is a minimum estimate and a rough approximation as the records are seldom complete and an unknown additional number of birds was probably released (e.g. Thomson, 1922). The number of years between first liberation and the time when the species was recorded to be abundant was taken as an indicator of the population growth rate immediately after introduction. When information for several widely separated introductions of the same species was available, they were all included separately acknowledging that they represent nonindependent observations. As the different populations of the same species are not statistically

independent, and the number of species for which data were available was small, no statistical tests were carried out.

Results

Variation across the native and introduced populations

Twenty-seven of the studied loci (*ACP*, *AK1-2*, *CK1-3*, *6PGD*, *GLUD*, *GOT1-2*, α -*GPDH1-2*, *GPI-3*, *EAP1-2*, *ES1* and 3, *ICD1*, *MDH2*, *NP*, *PGM1*, *SOD1-2*, *SDH* and *FL-ES*) were monomorphic in all introduced populations, whereas the remaining 13 loci were polymorphic in at least one of the samples (Table 2). Of these same loci, 22 were found to be polymorphic within the native range (Merilä *et al.* submitted; Table 3). Hence, the percentage of polymorphic loci in the introduced range ($P = 33.0$ per cent) was significantly lower than in the native range ($P = 55.0$ per cent; $\chi^2_1 = 4.11$, $P = 0.042$), as was the mean number of alleles per locus (Table 3; Fig. 2). The alleles which occurred in the native range but not in the introduced range tended to segregate in lower frequencies (Table 4) and occurred in significantly fewer populations than alternative alleles which were present in both ranges (Table 4). A logistic regression analysis revealed that the average frequency of the alternative allele in native populations was a poor predictor of its presence or absence in introduced populations (Table 5). However, the presence or absence of a particular allele was well predicted by the number of native populations in which it occurred (Table 5).

Three alleles present in New Zealand samples were not found in our survey of European populations. These alleles (at loci: *ACON1-2* and *GPI*) were found in one or two populations only and segregated at low frequencies ($q < 0.05$; Table 2).

Variation within introduced populations

Levels of genetic variation within all seven introduced populations were low (Table 6), and there were no significant differences in mean percentage (99 per cent criterion) polymorphic loci ($\chi^2_6 = 4.91$, $P = 0.56$), mean number of alleles per locus (Kruskal-Wallis; $H_6 = 4.70$, $P = 0.58$) or average heterozygosity (Kruskal-Wallis; $H_6 = 4.43$, $P = 0.62$) between the different introduced populations (Table 6). Likewise, levels of within-population genetic variability did not differ between native and introduced populations as revealed by comparison of mean

Table 2 Allele frequencies at 13 polymorphic loci in introduced greenfinch populations in New Zealand

Locus	Allele	Population and sample size						
		WEL 29	AUC 42	TAU 15	NAP 42	HOP 40	EYE 39	KAR 39
<i>ACON1</i>	<i>A</i>	1.000	1.000	1.000	1.000	0.962	1.000	1.000
	<i>B</i>					0.038		
<i>ACON2</i>	<i>A</i>	0.983	1.000	1.000	1.000	1.000	1.000	1.000
	<i>C</i>	0.017						
<i>ES2</i>	<i>A</i>	0.810	0.929	0.833	0.857	0.700	0.859	0.795
	<i>B</i>	0.086	0.024		0.036	0.063	0.026	0.051
	<i>C</i>	0.103	0.048	0.167	0.107	0.237	0.115	0.154
<i>G6PDH</i>	<i>A</i>	0.845	0.929	1.000	0.940	0.863	0.731	0.756
	<i>B</i>	0.138	0.048		0.048	0.125	0.205	0.231
	<i>C</i>	0.017	0.024		0.012	0.013	0.064	0.013
<i>ICD2</i>	<i>A</i>	0.983	0.988	1.000	1.000	1.000	0.987	0.962
	<i>B</i>	0.017	0.012				0.013	0.038
<i>LDH1</i>	<i>A</i>	0.983	1.000	1.000	1.000	1.000	1.000	1.000
	<i>B</i>	0.017						
<i>LDH2</i>	<i>A</i>	0.983	1.000	1.000	1.000	1.000	1.000	1.000
	<i>B</i>	0.017						
<i>MDH1</i>	<i>A</i>	1.000	1.000	1.000	1.000	1.000	1.000	0.987
	<i>B</i>							0.013
<i>MPI</i>	<i>A</i>	1.000	1.000	1.000	0.988	1.000	1.000	1.000
	<i>B</i>				0.012			
<i>PEPA</i>	<i>A</i>	0.983	1.000	1.000	0.964	0.988	1.000	0.974
	<i>B</i>	0.017			0.036	0.013		
	<i>C</i>							0.026
<i>PEPB</i>	<i>A</i>	0.810	0.810	0.867	0.833	0.913	0.795	0.744
	<i>C</i>	0.190	0.190	0.133	0.071	0.087	0.192	0.256
	<i>D</i>				0.095		0.013	
<i>GPI</i>	<i>A</i>	0.966	0.976	0.967	0.988	0.988	0.974	0.987
	<i>C</i>	0.017	0.024		0.012	0.013	0.026	0.013
	<i>D</i>	0.017		0.033				
<i>PGM2</i>	<i>A</i>	0.948	0.917	1.000	0.952	0.988	0.936	0.987
	<i>B</i>	0.052	0.083		0.048	0.013	0.064	0.013

Table 3 Number of populations (n_{POP}), number of alleles (A_{TOT}), mean number of alleles per locus (A), and number of polymorphic loci (P_{TOT}) in introduced and native populations of greenfinch. Note that this comparison was made pooling all individuals in the respective ranges and using each locus ($n = 40$) as a unit of comparison

Range	n_{POP}	A_{TOT}	A	P_{TOT}	Polymorphic loci (%)
Introduced	7	58	1.45 ± 0.11	13	33%
Native†	14	70	1.75 ± 0.14	22	55%
			$z\ddagger = 3.0^{**}$		$\chi^2_1 = 4.11^*$

†Data from Merilä *et al.* (1996), ‡Mann–Whitney.* $P < 0.05$, ** $P < 0.01$.

Fig. 2 The distribution of 40 loci with different numbers of alleles in native and introduced ranges of the greenfinch, *Carduelis chloris*. The proportion of monomorphic loci is significantly higher in introduced than in native populations ($\chi^2_1 = 4.11$, $P < 0.05$). Data for European populations from Merilä *et al.* (1996).

Table 4 Average frequency and average number of greenfinch populations containing alternative (all but the most common allele) alleles in the native range that were present or absent in the introduced range

	Allele frequency (mean $\pm$ SE)	No. of populations occupied (mean $\pm$ SE)	<i>n</i>
Present	0.051 $\pm$ 0.013	5.5 $\pm$ 1.21	16
Absent	0.022 $\pm$ 0.006	1.5 $\pm$ 0.17	14
<i>z</i> †	1.69	2.29	
<i>P</i>	0.09	0.02	

†Mann–Whitney.

Table 5 Logistic regression of presence/absence of alternative alleles in the introduced range as a function of their average frequency (Model I) and distribution (= number of populations containing them; Model II) in the native range. Model III considers both factors simultaneously. *R*, Standardized regression coefficient. *LR*, log likelihood ratio. Per cent correct, percentage of correctly classified cases

	Variable	<i>R</i>	$-2 \log LR$	<i>P</i>	Per cent correct
Model I	Frequency	−0.07	3.78	0.052	66.7%
Model II	Distribution	−0.21	10.95	<0.001	80.0%
Model III	Frequency	0.00	0.56	0.45	73.3%
	Distribution	−0.21	7.74	0.005	

percentage polymorphic loci, mean number of alleles per locus or average heterozygosity (Table 7).

Conformation to Hardy–Weinberg expectations

A straightforward prediction, under the assumption that introduced greenfinches have suffered from severe inbreeding and genetic drift, is that there should initially be greater departures from random-mating genotype frequencies in introduced than in native populations (e.g. Berlocher, 1984). However, significant ($P < 0.05$) departures from Hardy–Weinberg equilibrium were detected in only five of the 31 possible tests, and all were heterozygote deficiencies (not significant after sequential Bonferroni correction: $P > 0.05$). Furthermore, comparison of F_{IS} values among introduced (0.124) and native (0.133) populations gave no indication of a greater degree of inbreeding in the introduced populations.

The number of alleles segregating at low frequencies ($q \leq 0.05$) was very similar to the value to be expected according to a null-model (Table 8). Only in one population (Taupo) did the observed frequency differ from the expected one, but after sequential Bonferroni correction, this difference was not significant (Table 8).

Differentiation among introduced populations

The degree of differentiation among introduced populations was weak as allele frequencies in only four out of 13 polymorphic loci were significantly heterogeneous (*G6PDH*, *PEPB*, *ES2*, *ACON1*; exact tests; $P < 0.05$). Accordingly, F_{ST} as estimated by theta (θ) was small ($\theta = 0.022$) and almost two times lower than among native populations ($\theta = 0.041$). The F_{ST} estimate based on G_{ST} was somewhat higher ($G_{ST} = 0.033$) but, again, considerably lower than the estimate for native populations ($G_{ST} = 0.090$).

Table 6 Genetic variability measures for 40 loci in seven introduced greenfinch populations in New Zealand

Population	<i>n</i>	<i>A</i> ± SE	Polymorphic loci (<i>P</i>)		Heterozygosity (± SE)	
			≤ 95%	≤ 99%	Observed (<i>H</i> _O)	H-W expected† (<i>H</i> _E)
Auckland	42.0	1.20 ± 0.08	10.0	15.0	0.020 ± 0.011	0.020 ± 0.010
Taupo	15.0	1.08 ± 0.04	5.0	7.5	0.010 ± 0.006	0.015 ± 0.009
Napier	42.0	1.25 ± 0.09	7.5	17.5	0.020 ± 0.009	0.022 ± 0.010
Wellington	29.0	1.33 ± 0.10	10.0	25.0	0.023 ± 0.009	0.031 ± 0.013
Hope	40.0	1.23 ± 0.08	7.5	17.5	0.022 ± 0.012	0.025 ± 0.013
Eyrewell	39.0	1.23 ± 0.09	10.0	15.0	0.027 ± 0.014	0.030 ± 0.015
Karitane	39.0	1.25 ± 0.09	7.5	20.0	0.029 ± 0.014	0.033 ± 0.016
Mean	35.1	1.22 ± 0.03	8.2 ± 0.71	16.8 ± 2.02	0.023 ± 0.003	0.025 ± 0.002

A, mean number of alleles per locus; *n*, mean sample size per locus.
†Nei's unbiased estimate (Nei, 1978).

Table 7 Comparison of genetic variability in native European and introduced New Zealand populations of greenfinch

Population	<i>n</i>	% Polymorphic loci	Mean number of alleles/locus	Average expected heterozygosity
Native	14	14.1 ± 0.87%	1.19 ± 0.02	0.025 ± 0.002
Introduced	7	16.8 ± 2.02%	1.22 ± 0.03	0.025 ± 0.002
<i>z</i> †		1.58	1.13	0.64
<i>P</i>		0.11	0.26	0.52

n, number of populations.
†Mann–Whitney.

Fig. 3 Genetic similarity among introduced (New Zealand) and native (Europe and North Africa) populations of greenfinches as revealed by Neighbour-Joining analysis of Rogers's (1972) genetic distances. Data for European populations from Merilä *et al.* (1996).

Comparison of *F*_{ST} estimates and Rogers's genetic distances between North (*G*_{ST} = 0.02; *D*_R = 0.01) and South (*F*_{ST} = 0.022, *D*_R = 0.01) islands revealed similar levels of differentiation within both islands.

Despite the low degree of differentiation, there was a weak geographical component to differentiation as both *F*_{ST} (*r*_M = 0.69, *n* = 21, *P* = 0.003) and Rogers's genetic distances (*r*_M = 0.47, *n* = 21, *P* = 0.028) were

positively correlated with geographical distance separating pairs of introduced populations. Furthermore, cluster analysis of Rogers's genetic distances using the Neighbour-Joining algorithm indicated some correspondence between geography and genetic similarity as all New Zealand populations clustered together (Fig. 3). This figure further shows that the branch lengths in New Zealand are equivalent to those in northern Europe, indicating roughly similar levels of differentiation within introduced and native ranges (Fig. 3).

Comparative analyses

The seven species for which genetic variability has been assayed both in introduced and native populations, together with summary statistics, are listed in Table 9. Clearly, introduced populations do not

consistently have lower numbers of alleles per locus, fewer polymorphic loci, or lower average heterozygosities. In fact, the opposite is true in a number of instances (Table 9).

Data about the history of introduction — number of introduced individuals, number of introductions, and number of years lapsed between release and abundance — are presented in Table 9. The relationships between the differences in levels of genetic variability and parameters that are supposed to reflect population history are illustrated for native and introduced populations in Fig. 4. The 'reduction' in levels of genetic variation, whether measured by mean number of alleles per locus, percentage polymorphic loci, or average heterozygosity, shows a negative association with 'founder population size' and with number of introductions, and a positive association with the time to become described as abundant (Fig. 4).

Table 8 Expected and observed numbers of rare alleles in different introduced greenfinch populations

Population	Number of rare alleles		R^2	P
	Expected	Observed		
Wellington	7.1	8	0.110	0.97
Auckland	4.8	6	0.298	0.78
Taupo	2.9	1	1.274	0.02
Napier	5.3	7	0.543	0.77
Hope	6.0	5	0.170	0.80
Eyrewell	7.3	4	1.457	0.27
Karitane	8.0	5	1.138	0.42

Discussion

Variation within introduced populations

We found no evidence for a severe reduction in genetic variability in greenfinch populations introduced into New Zealand as compared to that observed in native European populations. Some alleles found in the native range were missing from the New Zealand populations, but this may be attributable to the more restricted scope of sampling of original source populations, as compared to the range of native populations included in this study, rather than to any effects of the introduction *per se*. Furthermore, as a consequence of inbreeding and

Fig. 4 Differences in levels of genetic variability between native and introduced populations of birds as a function of (A1-A3) number of introduced individuals, (B1-B3) time elapsed between introduction and expansion and (C1-C3) number of introductions. See Table 9 for details. The lines are linear regressions.

Table 9 Comparison of genetic variability in native and introduced bird populations

Species	Area†	n_p	n_i	Genetic parameters			Introduction history			
				A	P	H_E	Intro.	Abun.	n	#times
<i>Sturnus vulgaris</i> ¹	Native	6	24.0	1.42	28.5	0.033	—	—	—	—
	NZ	6	24.0	1.35	33.8	0.043	1867	1878	653	13
<i>Acridotheres tristis</i> ²	Native	7	28.4	1.43	31.5	0.060	—	—	—	—
	NZ	5	57.4	1.24	19.5	0.042	1862	1912	100	2
	AU	2	43.9	1.30	23.1	0.060	1862	1883	252	5
	HA	1	37.6	1.20	18.0	0.060	1865	1879	—	—
	FI	2	25.9	1.30	20.6	0.050	—	—	—	—
	SA	2	38.3	1.15	12.9	0.030	1888	1930	6	2
<i>Fringilla coelebs</i> ³	Native	10	28.7	1.34	24.5	0.048	—	—	—	—
	NZ	8	26.1	1.39	28.3	0.066	1867	1870	377	13
<i>Carpodacus mexicanus</i> ⁴	Native	6	40.6	1.48	27.4	0.045	—	—	—	—
	E-USA	6	38.5	1.37	23.7	0.042	1940	—	—	—
	HA	4	20.0	1.33	21.0	0.055	186?	—	—	—
<i>Carduelis chloris</i> ⁵	Native	14	33.6	1.19	14.1	0.025	—	—	—	—
	NZ	7	35.1	1.22	16.8	0.025	1862	1868	64	5
<i>Passer montanus</i> ⁶	Native	2	55	1.45	33.3	0.098	—	—	—	—
	USA	3	83	1.33	23.9	0.078	1870	—	20	1
	AU	1	15	1.3	23.1	0.074	1863	—	—	—
<i>Passer domesticus</i> ^{7‡}	Native	14	60.7	1.63	36.9	0.097	—	—	—	—
	NZ	4	51.4	1.42	36.2	0.080	1859	1869	418	7
	AU	3	36.7	1.50	33.3	0.103	1863	1906	494	8

¹Ross, 1983; ²Baker & Moeed, 1987; ³Baker, 1992, Baker *et al.*, 1990; ⁴Vásquez Phillips, 1992; ⁵This study; ⁶St. Louis & Barlow, 1988; ⁷Parkin & Cole, 1985.

n_p , number of populations; n_i , average number of individuals scored per locus; A , average number of alleles per locus; P , percentage polymorphic loci; H_E , average expected heterozygosity; n , minimum number of introduced individuals; #times, minimum number of introductions.

†AU, Australia; E-USA, Eastern USA; FI, Fiji; HA, Hawaii; NZ, New Zealand; SA, South Africa.

‡Only loci scored in all populations included.

drift, severe bottlenecks should also reduce average heterozygosity and elevate the inbreeding coefficient (F_{IS}), but none of these expectations was met in our data. This agrees with the fact that several plant (e.g. Burdon & Brown, 1986; Warwick *et al.*, 1987; Molina-Freaner & Jain, 1992; Novak & Mack, 1993) and animal (e.g. Ross, 1983; Baker, 1992) studies have found little or no reduction in the levels of within-population genetic variability in introduced populations when compared to native ones. Other studies have demonstrated reduced levels of variability in introduced populations (plants: Clegg & Brown, 1983; Glover & Barret, 1987; insects: Bryant *et al.*, 1981; reptiles: Taylor & Gorman, 1975; birds: Baker & Moeed, 1987; St. Louis & Barlow, 1988).

Theory predicts that loss of genetic variation as a result of bottlenecking is heavily dependent on the initial (effective) population size and the length of the time the population remains small (Nei *et al.*, 1975; Maruyama & Fuerst, 1985). The smaller the

initial population size, and the longer it remains small, the more variation will be lost. Furthermore, the genetic diversity of founding populations will probably increase as the number of introductions from source population(s) increases (Wade & McCauley, 1988; Whitlock & McCauley, 1990). As it is well known that the numbers of founders, as well as the numbers of introductions, have differed between species (e.g. Long, 1981), differing amounts of reduction in genetic variability are to be expected. Furthermore, different species differ in their ecology and life history characteristics and, hence, in their potential for rapid increase in population size following the introduction. The introduction of the greenfinch to New Zealand involved several releases in multiple locations (Long, 1981), and the initial increase in population size was so rapid that after only three years from the first release the species was considered so well established that 'further introductions were unnecessary' (Lever, 1987). Thus,

this rapid increase may have minimized the effects of inbreeding and genetic drift, and thereby prevented loss of alleles and reduction in heterozygosity (Nei *et al.*, 1975). Furthermore, even if an initial reduction in genetic variation had taken place locally, it could have been quickly restored by gene flow from other populations (Polans & Allard, 1989) — an outcome which might have applied to greenfinches as a result of multiple introductions in different parts of New Zealand.

Methodological considerations

A general problem in measuring the effects of bottlenecks is that levels of genetic variation before the bottleneck are rarely known (but see Gallardo *et al.*, 1995). Studies of introduced populations are powerful in the sense that prebottleneck levels of variation can be inferred with reasonable accuracy from levels estimated in source populations. Such a calibration is much more reliable than comparison to other outbred species (Taylor *et al.*, 1995), although it is based upon the assumption that source populations have not passed through bottlenecks after the introduced stock was split off. Another important assumption is that levels of variability have been estimated with similar accuracy in both native and introduced populations. As the number of alleles detected is strongly dependent on sample size, less extensive sampling in introduced populations would tend to bias their variability estimates downwards. However, sample sizes from introduced populations (Table 9) are often even larger than those for native populations. In our own study, sample sizes were similar for introduced and native populations (Table 9), and hence, the main conclusion about the lack of reduction in genetic variation following the bottleneck would not be changed even if additional sampling had increased the number of detected alleles.

The source population for the greenfinches introduced to New Zealand was probably from Britain. Although we do not know the genetic parameters of this ancestral stock, we have no reason to suspect that they would differ greatly from those of other northern European populations (Merilä *et al.*, 1996). This is because catching a large number of greenfinches is best undertaken outside of the breeding season when large flocks can be attracted to baits. If the greenfinches to be used for the introductions were captured in Britain during the nonbreeding season, many overwintering Scandinavian birds are quite likely to have been included in the samples. Furthermore, the genetic differences among

different northern European populations are very small (Merilä *et al.*, 1996) and, hence, allele frequencies in British populations are likely to be similar to those elsewhere in northern Europe. However, the presence of a few alleles in New Zealand samples that were not found in 14 native European populations (472 genomes) might derive from British populations which were not included in the previous survey (Merilä *et al.*, 1996).

Population divergence in New Zealand

Several studies have found that introduced species have evolved a stronger population structure in the introduced range as compared to the native range (e.g. Berlocher, 1984; Baker & Moeed, 1987; Baker & Dennison, 1991). This is to be expected if the influence of genetic drift has been prominent in creating allele frequency differences among different populations, and if gene flow between different introduced populations has been restricted. We found that New Zealand populations of greenfinches are only weakly structured and in fact exhibit lower or similar levels of spatial differentiation compared with native populations in Europe. However, given that the size of the native range (average distance between sampling localities [$\pm$ SE] = 1830 ± 180 km) is much larger than the size of the New Zealand range (495 ± 60 km), this indicates that in ≈ 100 years, a population structure in New Zealand has evolved comparable to that in Europe. Actually, if we correct for differences in sampling range by calculating residuals from the regression of Rogers's genetic distances on the geographical distance separating pairs of populations (for combined New Zealand and European data), and compare these among native and introduced populations, there is a tendency for introduced populations to be more differentiated (Mann–Whitney: $z = 1.80$, $n = 112$, $P = 0.07$).

Comparison with earlier studies

A review of the literature on genetic variability in introduced bird species revealed that in many instances, there is little or no evidence for reduced levels of genetic variability in introduced populations (e.g. Ross, 1983; Parkin & Cole, 1985; Baker, 1992; Vásquez-Phillips, 1992; Table 9). However, when analysed across the different studies, the degree of reduction in genetic variability seems to be inversely related to the size of the founding population and to the number of introductions (Fig. 4). Furthermore, the amount of variation lost seems to increase with

the length of time during which the population size remained small (Fig. 4). Hence, these trends suggest, in accordance with theory (Nei *et al.*, 1975), that only the most severe bottlenecks may result in loss of genetic variability. Hence, in spite of the small sample sizes, errors involved with estimation of genetic parameters and in particular, the very approximate nature of the demographic parameters (see methods), these data strongly suggest an association between demographic and genetic characteristics in founding populations.

There is an additional problem concerning not only this study, but the inference based on genetic studies of introduced populations as a whole. Studies of introduced populations are not based on any experimental design, and may therefore represent a non-random selection of introduced species and populations. For example, introduced bird species for which genetic data are available probably are not a random sample with respect to their ecology and life-history characteristics. Many species have been introduced, but only a minority have become successful colonists. For example, a total of 133 species of birds have been introduced into New Zealand, but only 38 of these have become successful colonists. Thirteen (34 per cent) of these successful ones are passerines (mostly multi-brooded) which are characterized by relatively large clutch sizes and high dispersal capacities. Hence, the fact that many studies have documented slight or no decrease in levels of genetic variability in introduced bird populations may to some degree reflect biased sampling rather than a small average effect of introductions. For example, small propagules, where reduction in genetic variance is most likely to be found, are also in greater peril of extinction due to demographic stochasticity. Hence, the general failure to detect reduced levels of genetic variability in introduced bird populations may not be solely attributable to methodological problems, but more likely, to the favourable demographic characteristics of these successfully introduced species which have made the genetic studies possible.

Conclusions

Our study provides another example of an introduced species suffering no demonstrable reduction in genetic variability following the population bottleneck that any introduction inevitably entails. Similar results have been obtained for other introduced bird populations (Ross, 1983; Baker, 1992), all of which seem to be characterized by a relatively large size of founding population, multiple introductions and

rapid population growth rate following the introduction. Thus, these are demographic properties that increase the probability of an introduction — whether desirable or undesirable — becoming a success.

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