ORIGINAL ARTICLE

Extensive linkage disequilibrium in a wild bird population

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Knowledge about the extent and patterns of linkage disequilibrium (LD) can provide important insights into demographic processes and strategies to identify the genetic basis of complex phenotypes in wild populations. However, data on the extent and patterns of LD from non-model vertebrate species from the wild are still scarce. We conducted so far the most extensive and detailed examination of LD in a pedigreed wild bird population using genotypes from 97 autosomal and 6 gonosomal microsatellites and a recently established linkage map of Siberian jays (*Perisoreus infaustus*). Analysis of syntenic marker pairs showed high levels of LD that extended over tens of centimorgans or several megabases and generally decayed as an increasing function of intermarker distance. In addition, significant LD was also very common between nonsyntenic markers. Patterns of LD varied across different linkage groups possibly because of the

differences in chromosomal structure (macro-, micro-, and Z-chromosome). In particular, the level of LD was significantly lower on the Z-chromosome than on the autosomes at comparable genetic distances. In general, the high levels and extent of LD in this population are likely owing to its relatively small size, significant intrapopulation genetic structure, and occurrence of inbreeding. Whatever the cause, the long-range LD between syntenic loci suggests that LD mapping of phenotypic traits in this population using low-density markers maps is feasible. However, the frequent occurrence of LD between nonsyntenic markers suggests that the combined use of linkage and LD methods is needed to reduce the likelihood of false-positive associations between marker loci and traits of ecological and evolutionary interest.

Heredity (2010) **104**, 600–610; doi:10.1038/hdy.2009.150; published online 4 November 2009

Keywords: genome wide; linkage disequilibrium; Siberian jay; microsatellites

Introduction

The levels and extent of linkage disequilibrium (LD) are affected by many factors, including population size and structure, mating pattern, selection, genetic drift, migration, admixture, mutation, gene conversion, epistasis, and recombination (Ardlie *et al.*, 2002). Analysis of LD can thus give insights into demographic history and dynamics of a given population. It has been shown to be a very powerful approach to distinguish between alternative models (for example, the hitchhiking vs the background selection models) of genome evolution (Lewontin, 1964). In addition, information about the patterns of LD can be used for mapping genes or quantitative trait loci (QTL), particularly in species where traditional pedigree-based QTL-mapping approaches are not feasible as is the case with many wild species (see Rafalski and Morgante, 2004).

There has been a number of investigations about the levels and extent of LD in humans (for example, Stephens *et al.*, 2001), domesticated animals (for example, Farnir *et al.*, 2000), plants (for example, Remington *et al.*, 2001), and model organisms such as *Drosophila* (for example, Nordborg *et al.*, 2002). In addition, LD has also

been used successfully and extensively to investigate the evolutionary history of certain bacteria of zoonotic importance (for example, Wilson *et al.*, 2008). However, detailed studies to the effect of LD from wild vertebrate populations are confined to a few studies of birds (Edwards and Dillon, 2004; Backström *et al.*, 2006; Balakrishnan and Edwards, 2009) and mammals (McRae *et al.*, 2005; Laurie *et al.*, 2007; Slate and Pemberton, 2007). However, many key questions in evolutionary biology and genetics cannot be addressed using model systems or domesticated populations (for example, Gracey and Cossins, 2003), and studies of wild populations can provide important insights into evolutionary genetics not traceable in the laboratory or captivity (for example, McRae *et al.*, 2005; Ellegren and Sheldon, 2008). Thus, the measures of LD may well find their application in future studies of wild populations, in which there is considerable current interest in linkage and QTL mapping and the subsequent study of the genetics of traits of ecological importance (for example, Backström *et al.*, 2006; Gratten *et al.*, 2008; Stapley *et al.*, 2008). However, studies of LD in wild populations made so far are characterized by a limited number of analyzed chromosomes/linkage groups (LGs). However, the information on the structure of LD at the genome level is crucial for determining appropriate experimental design and guiding the selection and spacing of markers to create marker maps useful in candidate gene, candidate region, and eventually whole-genome association studies (De La Vega *et al.*, 2005).

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Received 6 May 2009; revised 21 September 2009; accepted 25 September 2009; published online 4 November 2009

The overall extent of LD across the genome is one of several critical attributes, which affects how readily phenotypic traits in natural populations can be mapped using whole-genome association studies (Flint-Garcia *et al.*, 2003). On the one hand, extensive LD allows associations to be detected more readily using a small number of distantly placed markers, whereas low LD necessitates fine-scale mapping. On the other hand, long extending LD is a potential barrier to the localization of causative polymorphisms of phenotypic effects, whereas gene identification might be less tedious in the case of limited LD. Data from humans, livestock, and selfing plants have shown a general pattern of decaying LD with physical/recombination distance between markers. However, the rate of this decay varies among species, populations, and different parts of the genome (see Kolkman *et al.*, 2007; Slate and Pemberton, 2007).

The aim of this study was to investigate the magnitude and extent of LD in the genome of a wild population of Siberian jays (*Perisoreus infaustus*) making use of 103 microsatellite markers genotyped in 349 individuals of 5 major families. We examined and compared the strength of pairwise LD between syntenic and non-syntenic markers using three different measures of strength of association. In particular, we were interested in learning how far—in terms of centimorgans—the significant linkage in the Siberian jay genome extends. By extrapolating the LD measured on the genome scale, this study aimed to explore the prospects and strategies for LD-based association mapping in a wild bird population.

Materials and methods

Study species, study population, and pedigree

The Siberian jay (*P. infaustus*) is a relatively long-lived (average generation time ca. 4 years) oscine passerine bird from the Corvidae family. It has a stable socially monogamous breeding system in which lifelong pair bonds are formed in permanent territories established in the coniferous forest of the northern Eurasian taiga (Ekman *et al.*, 1994). Siberian jays live in small territorial groups (3–5 individuals) consisting of a breeding (alpha) pair, their retained offspring, and/or unrelated immigrants (Ekman *et al.*, 1994). The species is also highly sedentary and philopatric so that closely related males often hold neighboring territories (Ekman *et al.*, 2001).

A long-term field study of the Siberian jay (*P. infaustus*) population in Suupohja (ca. 66°18'N, 29°29'E) in Western Finland has been conducted since 1974. The study population, field methods and the data structure are described in detail by Lillandt *et al.* (2001, 2003), Alho *et al.* (2009) and, in particular, Jaari *et al.* (2009). The study population is in a semi-isolated forest area near the southwestern border of the species' breeding range in Finland, where its population density is much lower than within the continuous main distribution area in the north (Kemppainen, 2002). Siberian jays were monitored mainly in three neighboring forest areas, separated from each other by 100–1500 m wide agricultural fields or peatlands.

The pedigree, hereafter the Suupohja pedigree, used in this study was built based on field observations (Lillandt *et al.*, 2001) and molecular tools as described in Jaari *et al.*

(2009). The pedigree is composed of five major families consisting of 349 animals and was from a large Siberian jay data set comprising more than 1000 individuals sampled in 1975–2006. The five family pedigrees varied in depth from four to six generations and ranged in size from 65 to 73 animals. These family pedigree configurations yield large numbers of pairwise relationships, which include, but are not limited to, parent–offspring, full-sib, half-sib, grandparent–grandchild, great grandparent–great grandchild, avuncular, half-avuncular, and first cousin relationships. A more thorough description of the structure of the mapping pedigree can be found from Lillandt *et al.* (2001, 2003) and Jaari *et al.* (2009).

As the levels and extent of LD are expected to be higher among closely related individuals than among more distantly related individuals (for example, Slate and Pemberton, 2007), we also analyzed LD in a restricted data consisting on the founders of pedigree data. To this effect we confined our analyses (see below) to those 98 individuals that were assumed to be unrelated in the first two or three generations in the five family pedigrees. We reasoned that if the patterns and extent of LD are roughly similar between analyses based on pedigree and founder data, then the results of the pedigree analyses can be generalized to the whole population data.

Microsatellite markers and genotypes

The pedigree has been genotyped in a total of 107 microsatellites comprising 101 autosomal and 6 Z-chromosomal markers (Jaari *et al.*, 2009). A genetic map has been constructed by following the co-segregation within the pedigree and determining marker order and distances with the software CRIMAP (Green *et al.*, 1990), described in detail in Jaari *et al.* (2009). The microsatellites were distributed in nine autosomal LGs (LG1–LG9) and one Z-chromosome LG (LGZ) covering 886.6 cM in total. Eight LGs (LGs 1–7 and Z) that contain three or more markers (3–29) were analyzed here, totalling 103 microsatellite markers. The length of LGs varied from 26.4 to 185.6 cM with the average intermarker distance ranging from 4.42 cM on LGZ to 17.02 cM on LG3 (Jaari *et al.*, 2009). The marker order and all pairwise intermarker genetic distances are based on the sex-averaged linkage maps of Siberian jay (see: <http://www.biomedcentral.com/content/pdf/1471-2164-10-1.pdf>).

Microsatellite variation and between-family genetic structure

Microsatellite variation was assessed by estimating the number of alleles and the observed heterozygosity using the Excel Microsatellite Toolkit version 3.1.1 (Park, 2001). Within the population, genetic differentiation among families was quantified by the θ estimator of F_{ST} (Weir and Cockerham, 1984). We also calculated the value of f , which corresponds to Wright's (1951) within-population inbreeding coefficient F_{IS} , across the loci over the total samples. These calculations were performed with FSTAT version 2.9.3.2 (Goudet, 2002) and the significance and the 95% confidence intervals of θ and f were determined by 10 000 permutations.

Measures of LD and statistical significance of association. The strength of LD was measured using two different metrics. First, we used Lewontin's normalized D' (Lewontin, 1964) modified for multiple alleles (Hedrick, 1987). This statistic has been used earlier to measure the extent of LD between markers spanning one single chromosome and/or the whole genome in humans (for example, Mohlke *et al.*, 2001; Ardlie *et al.*, 2002), livestock (for example, Farnir *et al.*, 2000; Khatkar *et al.*, 2006a, b), and wild vertebrates (for example, Backström *et al.*, 2006; Slate and Pemberton, 2007). Apart from facilitating the comparison of the results with those other studies, D' is a convenient measure of LD as it allows use of highly polymorphic markers and is less sensitive to variation in marker allele frequencies and marker information content than other measures of LD (Hedrick, 1987; McRae *et al.*, 2002). Following Hedrick (1987), LD between two multiallelic loci A and B was measured as

$$D' = \frac{\sum_{i=1}^u \sum_{j=1}^v p_i q_j |D'_{ij}|}{\sum_{i=1}^u \sum_{j=1}^v p_i q_j} \quad (1)$$

where u and v are the respective number of alleles at the two loci, p_i and q_i are the population frequencies of marker allele i at locus A and marker allele j at locus B, and $|D'_{ij}|$ is the absolute value of Lewontin's (1964) normalized LD measure computed as

$$D'_{ij} = \frac{D_{ij}}{D_{\max}}, \text{ with } D_{ij} = p_{ij} p_i q_j \quad (2)$$

where p_{ij} is the observed frequency of gametes $A_i B_j$, and p_i and q_i are the frequencies of allele A_i and B_j , respectively, and

$$D_{\max} = \begin{cases} \min[p_i q_j, (1 - p_i)(1 - q_j)]; & D_{ij} < 0 \\ \min[p_i(1 - q_j), (1 - p_i)q_j]; & D_{ij} > 0 \end{cases} \quad (3)$$

Second, we used the standardized χ'^2 (Yamazaki, 1977; Hedrick, 1987). Simulation studies (for example, Zhao *et al.*, 2005) have shown that χ'^2 is the preferred measure of LD for multiallelic markers for the purpose of QTL mapping, as it closely tracks the regression of allelic state at a QTL on allelic state at a multiallelic marker. Hence, it conveys the same information for multiallelic markers as r^2 (Hill and Robertson, 1968) for biallelic markers (Lipkin

et al., 2009). The measure r^2 is useful for biallelic markers, as it stands in inverse proportion to the sample size needed to show significance. However, when pooling across allelic pairs for multiallelic markers, r^2 weighted by the product of allele frequencies was found to be strongly undervalued (Heifetz *et al.*, 2005; Zhao *et al.*, 2005). Hence, the measure χ'^2 is considered here instead of r^2 , and χ'^2 was calculated as

$$\chi'^2 = \frac{\chi^2}{N(n-1)} = \sum_{i=1}^u \sum_{j=1}^v \frac{D_{ij}^2}{p_i q_j (n-1)} \quad (4)$$

where N is the population size, and n is the number of alleles at the marker with the smaller number of alleles, that is, $n = \min(u, v)$.

Multiallelic interallelic disequilibrium analysis software (MIDAS) (Gaunt *et al.*, 2006) provided estimates of D'_{ij} , p_i , q_i , and χ'^2 for allelic combinations; D' and χ'^2 for each marker pair were then calculated using equations (1) and (4) above. The graphical visualization of interallelic disequilibrium between alleles of the multiallelic microsatellites was also provided by the software. It can offer insights into patterns of LD across genomic regions and inferences about patterns of evolution and selection. In addition, the program has been shown to considerably increase both the statistical power and the accuracy of estimation of the strength of LD (Gaunt *et al.*, 2006).

In addition to MIDAS analyses, the statistical significance (P -value) of the observed association between pairs of alleles under the null hypothesis of random allelic assortment was also estimated by a Monte-Carlo approximation of Fisher's exact test as implemented in the software Arlequin (Schneider *et al.*, 2000) using a Markov chain extension to Fisher's exact test for $R \times C$ contingency tables (Slatkin, 1994). A total of 100 000 alternative tables were explored by the Markov chain and probabilities (P -values) were typically estimated with a standard error of <0.001 . No attempt was made to correct for multiple testing, as the large number of tests would result in very low statistical power (see McRae *et al.*, 2002).

The LD and P -values of significance were computed between all syntenic markers and between all pairs of nonsyntenic markers among the LGs from seven different data sets. Data set 1 comprised genotypes from the total Suupohja pedigree; data set 2 comprised genotypes from only the founders ($n=98$, Table 1);

Table 1 Linkage disequilibrium (LD) summary statistics of mean values of the Lewontin's normalized D' (Lewontin, 1964), the standardized χ'^2 (Yamazaki, 1977; Hedrick, 1987), and the proportion of marker pairs in significant ($P < 0.05$) LD in all linkage groups separately for syntenic and nonsyntenic markers

Data set	n	Metric	Syntenic					Nonsyntenic
			0–20 cM	20–40 cM	40–60 cM	60–100 cM	100–190 cM	
1 (The pedigree)	349	D'	0.517 (0.174)	0.484 (0.153)	0.476 (0.145)	0.381 (0.129)	0.308 (0.113)	0.354 (0.121)
		χ'^2	0.264 (0.098)	0.187 (0.074)	0.174 (0.072)	0.168 (0.067)	0.160 (0.058)	0.165 (0.061)
		$P < 0.05$	211/222 (0.95)	189/206 (0.92)	138/151 (0.91)	200/224 (0.89)	113/148 (0.76)	3567/4309 (0.83)
2 (Founders)	98	D'	0.483 (0.144)	0.462 (0.151)	0.456 (0.137)	0.364 (0.119)	0.282 (0.101)	0.362 (0.127)
		χ'^2	0.231 (0.087)	0.162 (0.067)	0.158 (0.058)	0.147 (0.054)	0.144 (0.052)	0.168 (0.065)
		$P < 0.05$	204/222 (0.92)	162/206 (0.79)	104/151 (0.69)	117/224 (0.52)	61/148 (0.41)	2213/4309 (0.51)

Values in parenthesis are standard errors for D' and χ'^2 and the percentages for $P < 0.05$. n is the number of individuals analyzed.

and data sets 3–7 comprised genotypes from the five largest families (Table 3), respectively. For the purpose of comparison to other studies, the measure D' , which has been used widely in studies of non-human vertebrates (for example, Edwards and Dillon, 2004; Backström *et al.*, 2006; Slate and Pemberton, 2007), was included in further analysis within and among the LGs. Plots of all pairwise comparisons among associations relative to genetic distances and the genetic diversity observed at the microsatellites, in addition to the frequency distribution of D' and cumulative frequency of Fisher exact P -values, were generated in Microsoft Excel. Analysis of variance was performed by SPSS release 15.0.1 statistical package and the Mantel test (10 000 permutations) was implemented in R package.

Results

Patterns of polymorphism and between-family genetic structure

A total of 35 557 autosomal and 661 Z-linked microsatellite genotypes in the sample were available for analysis. The average observed heterozygosity ($H_O = 0.56 \pm 0.003$) for autosomal markers was significantly higher than those for gonosomal markers ($H_O = 0.27 \pm 0.01$; Supplementary Figure S1). However, gonosomal markers were more differentiated (mean $F_{ST} = 0.111 \pm 0.021$) than the autosomal markers (mean $F_{ST} = 0.075 \pm 0.013$) among families (see Supplementary Figure S2). The genetic divergence among the families was high ($F_{ST} = 0.082$, 95% CI = 0.068–0.100), indicating substantial structure between the families. Among the individuals, the mean F_{IS} across all the loci was 0.036 (± 0.015) and deviated significantly ($P < 0.05$) from 0.

LD in syntenic markers in the pedigree

LD was estimated for 951 syntenic marker pairs. Figure 1 and Table 1 show the distribution of D' values as a function of genetic distance in centimorgans. In general, D' decayed with distance varying between 0.025 and 0.913 (see Figures 1 and 2) and the 'half-length' of LD (measured as the distance at which mean D' falls to 0.5) is ca. 20 cM (Figure 1). There was also extensive variability

in the magnitude of D' at a given genetic distance reflecting wide local variations in the extent of LD across the LGs. Marker distance was significantly and negatively correlated with D' for markers spaced < 60 cM apart (Mantel's test: $r = -0.22$, $n = 579$, $P < 0.001$), whereas markers separated by > 60 cM showed no significant relationship with distance (Mantel's test: $r = -0.014$, $n = 372$, $P > 0.1$). D' averaged 51.7% for marker pairs < 20 cM apart, decaying relatively moderately to ca. 38% at distances of 60 cM, and then leveling slowly below 30% for markers separated by 100 cM or more. However, significant ($P < 0.05$) and strong associations ($D' > 0.5$) were observed also for many of the comparisons among loci separated by > 100 cM (Table 1; Figure 1).

As compared with the LD measured by D' , use of the χ'^2 metric resulted in a 50% reduction in LD estimates (Table 1). In particular, there seems to be much more variation in the value of D' at a given genetic distance than in the values of χ'^2 (Figure 1). In contrast, the mean estimate of LD within LGs decays more rapidly with increasing genetic distances < 60 cM when measured with χ'^2 as compared to D' (Table 1; Figure 1).

LD was also further assessed within the LGs using the estimator D' . Table 2 summarizes the frequency distribution of D' against genetic distance for the eight LGs. The highest frequency of observed values of D' was between 0.40 and 0.60 for all LGs except LG7, whereas the highest and lowest D' values differed between the LGs (Table 2). The mean D' varied between 0.461 and 0.726, and the difference was highly significant among the LGs (one-way analysis of variance; $F_{7,973} = 6.46$, $P < 0.0001$; Table 2).

There was a great deal of interchromosomal heterogeneity in the way LD varied as a function of physical distance between markers (Figure 2). Despite that pairwise LD decays significantly over ca. 5–20 cM in the five autosomal LGs (LG1, LG2, LG5, LG6, and LGZ), marker pairs on autosomal LG3, LG4, and LG7 showed elevated LD over distances that approach the total length of the LGs. At comparable distances (0–5, 5–10, 10–15, 15–20, and 20–25 cM), D' values were systematically lower for the Z-linked marker pairs than for the autosomal marker pairs (data not shown; Figure 2). When logarithmic trend lines were fitted to the data of

Figure 1 Linkage disequilibrium as measured by D' and χ'^2 as a function of genetic distances (cM) between autosomal syntenic markers for the overall distance (longer curves) and > 60 cM (shorter curves). The dark and gray lines give exponential trend line fits of D' and χ'^2 , respectively.

Figure 2 Linkage disequilibrium (D') as a function of the genetic distances (cM) between marker pairs within the same LG. The second-order polynomial linear regression fits to the data of LG1, the logarithmic trend lines fit to the data of LG2, LG5, LG6, and LGZ; and the linear regression trend lines fit to the data of LG3, LG4, and LG7.

LG2, LG5, LG6, and LGZ, the coefficients of determination (R^2) vary from 0.035 for LG2 to 0.556 for LG6, whereas the second-order polynomial linear regression

fits best to the data of LG1 by the valid criterion of R^2 (Figure 2). All negative correlations between LD and genetic distances were significant ($P < 0.05$ for LG1, LG2,

Table 2 Distribution of D' against genetic distance within linkage groups

Linkage group (LG)	No. of loci	Length (cM)	LD (D') bin					Mean $D' \pm s.d.$
			0.00–0.20	0.20–0.40	0.40–0.60	0.60–0.80	0.80–1.00	
LG1	29	185.6	0.002	0.279	0.556	0.153	0.007	0.476 (0.122)
LG2	27	181.5	0.006	0.262	0.595	0.120	0.022	0.480 (0.132)
LG3	13	182.6	0	0.026	0.769	0.192	0.013	0.534 (0.076)
LG4	11	98.2	0	0.164	0.564	0.218	0.055	0.535 (0.144)
LG5	8	85.2	0	0.214	0.607	0.143	0.036	0.526 (0.124)
LG6	6	78.2	0	0.200	0.800	0	0	0.461 (0.071)
LG7	3	26.4	0	0	0.333	0.667	0	0.726 (0.161)
LGZ	6	23.8	0	0.125	0.75	0.125	0	0.501 (0.113)

Figure 3 Frequency distributions of D' for syntenic and nonsyntenic marker pairs.

LG5, LG6, and LG7), but the positive correlations for LG3, LG4, and LG7 were nonsignificant ($P > 0.05$) by the Mantel permutation test. Moreover, the 'half-length' of LD also varied among the LGs (ca. 20 cM for LG1, LG2, and LG6, ca. 30 cM for LG5, and ca. 5 cM for LGZ; see Figure 2).

Whether LD in chromosomes is structured in 'block-like' manner is of considerable interest as if it is so, it will simplify LD-mapping studies (Zhang *et al.*, 2002). Again, the patterns of LD measured by the D' estimates are very different among the LGs (Supplementary Figure S3). For example, in LG3 and LG4, there are much less and isolated associations between the syntenic markers, whereas in LG1 and LG2 the associations are patchy, not block like. On the other hand, pairwise combinations of alleles at the syntenic loci show considerable difference in magnitude, significance, and patterns of interallelic disequilibrium among the LGs. It is clear that some regions within the LGs have extended LD whereas others do not (Supplementary Figure S3). In addition, D' values were not distributed uniformly across the nonsyntenic LD map. The results indicated again extensive LD within LGs (see Supplementary Figure S3). However, because of the large number of comparisons, none would be individually significant if Bonferroni corrections were applied.

Examination of the cumulative distribution of P -values for syntenic marker pairs showed a great proportion of syntenic marker pairs (90.2%, 858/951) to be insignificant ($P < 0.05$) LD (Table 1; Supplementary Figure S4). Of the syntenic marker pairs separated by 20 cM or less, 95% (211/222) were insignificant ($P < 0.05$) LD. However,

substantial and significant ($P < 0.05$) LD were also observed among syntenic markers separated by > 60 cMs.

LD in nonsyntenic markers in the pedigree

Gametic LD determined for the 4309 nonsyntenic marker pairs and the distribution of D' values for nonsyntenic markers (0.091–0.824; Figure 3) overlapped strongly with that for syntenic markers (0.025–0.913; Figure 3), although nonsyntenic pairs did have a significantly lower mean D' (0.351 ± 0.121) than nonsyntenic pairs (mean $D' = 0.439 \pm 0.151$; $t_{5258} = 5.21$, $P < 0.001$). Likewise, a significant lower mean χ^2 was observed in pairs of nonsyntenic markers (0.165 ± 0.061) than that in syntenic pairs (mean $\chi^2 = 0.223 \pm 0.078$; $t_{5258} = 4.61$, $P < 0.001$).

We looked also for possible significant LD between nonsyntenic loci. Examination of the cumulative frequency distribution of P -values between nonsyntenic loci indicates that significant ($P < 0.05$) LD occurs in 82.8% (3567/4309) of the marker pairs. In addition, as shown in Table 1, the average D' value measured between nonsyntenic marker pairs was 0.354, therefore quite similar to the D' values (0.356) found for distant (> 60 cM) though syntenic loci. Hence, the data from the Siberian jay pedigree support the presence of significant long-range LD among nonsyntenic markers.

LD in the founders and the families

LD between syntenic and nonsyntenic markers was also examined in the founders as well as within the

Table 3 Linkage disequilibrium (LD) summary statistics of mean values of the Lewontin's normalized D' (Lewontin, 1964) against intervals of genetic distances within the five large families

Data set	n	Syntenic					Nonsyntenic
		0–20 cM	20–40 cM	40–60 cM	60–100 cM	100–190 cM	
3 (Family A)	70	0.554 (0.166)	0.492 (0.151)	0.487 (0.139)	0.421 (0.131)	0.356 (0.109)	0.382 (0.117)
4 (Family B)	65	0.532 (0.159)	0.499 (0.148)	0.491 (0.141)	0.436 (0.135)	0.371 (0.114)	0.387 (0.119)
5 (Family C)	69	0.523 (0.149)	0.486 (0.147)	0.478 (0.136)	0.409 (0.127)	0.366 (0.107)	0.369 (0.115)
6 (Family D)	72	0.534 (0.158)	0.489 (0.149)	0.481 (0.137)	0.411 (0.129)	0.373 (0.112)	0.376 (0.109)
7 (Family E)	73	0.519 (0.138)	0.486 (0.131)	0.475 (0.128)	0.391 (0.119)	0.325 (0.111)	0.343 (0.118)

n is the number of individuals analyzed.

five largest families (Tables 1 and 3). In general, LD between syntenic markers was lower in data set 2 (the founders) than in data set 1 (the whole Suupohja pedigree) either measured by D' ($F_{1, 951} = 9.31$, $P < 0.05$) or by χ^2 ($F_{1, 951} = 4.67$, $P < 0.05$). A larger proportion of marker pairs in significant LD were also observed in syntenic markers (3567/4309, 0.83) than in nonsyntenic markers (2213/4309, 0.51; Table 1). However, despite statistically significant differences in levels of LD in pedigree and founder data, the actual differences in LD levels were slight (Table 1). This together with the similar decay of LD with physical distance in the two data sets suggests that the extent and patterns of LD in the pedigree data reflect that in the whole population in general.

Table 3 shows the mean D' against different intervals of genetic distance for syntenic marker pairs as well as for nonsyntenic marker pairs in each family. The results indicate systematically higher LD for the within-family data than for the whole pedigree data both for syntenic and nonsyntenic marker pairs (Table 3). However, the actual numerical differences in the levels of LD between family and pedigree data are slight (see Tables 1 and 3). Furthermore, a similar rate of decay with genetic distance occurs between each family and the whole pedigree in terms of the mean D' (Tables 1 and 3).

Discussion

This work is so far the most comprehensive study of LD in a wild bird population. We discovered very high levels of LD across the genome of Siberian jays. LD extended for tens of centimorgans within LGs and occurred also among nonsyntenic markers suggesting extensive LD in the genome of the Siberian jay. However, LD did decay with genetic distance across the genome, and there was marked heterogeneity in the extent of LD among LGs. Nevertheless, as compared with other species for which information about the levels and extent of LD is available (for example, Edwards and Dillon, 2004; Backström *et al.*, 2006; Slate and Pemberton, 2007; Balakrishnan and Edwards, 2009), the patterns and levels of LD uncovered in this study are clearly exceptional. Although part of the explanation may reside on the fact that our data consisted to a certain extent of closely related individuals from large pedigreed families, the fact that the extent and patterns of LD were very similar irrespectively whether whole pedigree, founder, or family data were used suggests that the results reflect the 'true' situation in this population.

LD among syntenic markers

Considerable amounts of LD between loci were detected by both the D' and χ^2 -statistics. The large difference in the degree of LD indicated by these two measures was not unexpected (see the review by Slatkin, 2008) and the amount of LD decayed with increasing distance between markers for both metrics.

LD was found to occur for marker pairs separated by long genetic distances. The 'half-length' of LD that was chosen in this study was the same as previously used in LD studies of human (Reich *et al.*, 2001), cattle (Farnir *et al.*, 2000), dog (Sutter *et al.*, 2004), chicken (Andreescu *et al.*, 2007), and collared flycatcher (Backström *et al.*, 2006) populations. However, the extensive LD in the Siberian jay extended over much longer distances than that observed in the above-mentioned species. Assuming a similar mean recombination rate as in another passerine bird—the zebra finch (3.18 cM Mb⁻¹; Stapley *et al.*, 2008)—the 'half-length' of LD in Siberian jays is ca. 6.28 Mb (= 20 (cM)/3.18 (cM Mb⁻¹)) in physical distance. The 'half-length' of LD varied from 5 to 10 cM in the domestic livestock populations, whereas appreciable LD were detected only among markers separated by 1 cM (Andreescu *et al.*, 2007) or up to 5 cM (Heifetz *et al.*, 2005) in chicken. However, studies of wild bird populations have produced heterogeneous results to this effect. Edwards and Dillon (2004) found low levels of LD at small genomic scales in autosomes of red-winged blackbird *Agelaius phoeniceus*, whereas Backström *et al.* (2006) found relatively higher levels of LD with the half-length of less than 1 cM on the Z-chromosome of the collared flycatcher. Even lower levels of LD—even over as short distances as 300 bp—were detected in the zebra finch *Taeniopygia guttata* populations (Balakrishnan and Edwards, 2009). Nevertheless, these latter results were based on a low number of spaced markers. In the studies of other wild vertebrate populations, relatively high levels of LD extended over several tens of centimorgans in the global pattern of red deer (Slate and Pemberton, 2007), which are comparable with that found in the Siberian jay.

High levels of LD can be built up in small and subdivided populations because small effective size and admixture between genetically divergent lineages are expected to increase levels of LD (for example, Peltonen *et al.*, 2000). These factors may interactively influence the observed high levels of LD in the Siberian jays, which have undergone long-term isolation (Lillandt *et al.*, 2001, 2003) and within which significant inbreeding (Alho *et al.*, 2009; $F_{IS} = 0.036$, $P < 0.05$, this study) and population substructure ($F_{ST} = 0.082$, $P < 0.05$) have been found.

Although high levels of LD was observed in this study, direct comparisons with other studies should be done with caution because of the differences in measurement metrics, genetic map units, mating system, the targeted genomic region studied (for example, the MHC region studied in red-winged blackbirds (Edwards and Dillion, 2004)—a region that is known to have extremely high recombination rates in mammals (for example, Dumont and Payseur, 2008) and birds (for example, Kaufman and Salomonsen, 1997)) and the molecular markers used etc (see for example, Slate and Pemberton, 2007; Meadows *et al.*, 2008). Another potential caveat in the comparisons concerns the variance in relatedness among individuals in different data sets. For instance, as in case of the family data used here, recombination has not yet had much time to break up associations between loci and it is therefore very hard to compare the extent and level of LD observed here with that in other populations. As further indicated by the results from the different data sets, LD was significantly greater in the pedigree (data set 1) than the founders (data set 2), which were less related to each other (Table 1). In data set 1, the associations between markers, particularly the nonrecombinant ones, have been represented repeatedly among the descendants of a small number of highly successful individuals such as some of the founders (see Slate and Pemberton, 2007). However, high levels of LD were still observed in the founders, indicating that the inference is likely to apply to the whole population too. As the pedigree is part of a larger data set, some of the 'founders' might be still related if the pedigree of the whole data set could be resolved. In addition, the behavior of LD—as measured by the D' metric—was found to differ between the families being systematically higher within the families than in the whole pedigree. This could be explained by the closer relatedness between the family samples (c.f. most of them are full-sibs) than that between samples from the whole pedigree, although the effect of population structure on LD may have been reduced. The persistence of LD over long distances within the families is therefore not surprising. A small effective population size and closely related individuals may lie behind the high LD observed in this study.

Variation in LD among LGs

Our results showed considerable variation in LD not only between Z-chromosomal and autosomal LGs, but also among different autosomal LGs (Figure 2). Similar interchromosomal heterogeneity in LD among syntenic marker pairs has been observed also in rice (Mather *et al.*, 2007), pigs (Nsengimana *et al.*, 2004), and red deer (Slate and Pemberton, 2007). Differences in local recombination rates may account for this heterogeneity (see Yu *et al.*, 2001). However, the effect should be slight as the local recombination rate always varies at shorter scales than what can be detected by markers separated by a few kilobases (see for example, Pritchard and Przeworski, 2001), rather than several and even tens of centimorgans as in this study. Another potential explanation could be the 'Bulmer effect' describing a higher LD between genomic regions harboring QTL undergoing selection (Bulmer, 1971). Despite the fact that D' is assumed to be a frequency-independent

measure (Hedrick, 1987), the significant linkage-group effect could still be attributable to heterogeneity in number of marker pairs in LGs (Slate and Pemberton, 2007). Likewise, the heterogeneous karyotype and chromosomal structure of birds with few macrochromosomes and many microchromosomes (Balakrishnan and Edwards, 2009) could at least partly explain our finding.

When examining the relationship between the LD and genetic distance, a progression of decreasing association, measured with the D' metric, was not always observed in the different LGs (Figure 2). This observation parallels that described in humans (Jeffreys *et al.*, 2001), barley (Caldwell *et al.*, 2006), and zebra finch (Balakrishnan and Edwards, 2009). One plausible explanation for the tendency of LD to increase with physical distance is the presence of contrasting locus histories within the same LG. The relationship between LD and genetic distance might also be shaped by gene conversion. For example, for closely linked markers, LD may also be broken up by gene conversion (see Pritchard and Przeworski, 2001). Indeed, gene conversion has been suggested to be quite frequent in the Siberian jay, although the precise details are unknown (Jaari *et al.*, 2009). Another explanation for difference in rate of decay in LD between LGs is that the precision of linkage map distances differs between markers and LGs because of number of markers used, marker variability, and genotyping errors (see Jaari *et al.*, 2009).

LD on the Z-chromosome

The levels of polymorphism and LD for microsatellites were found to be significantly lower in Z-chromosome than in autosomes at comparable genetic distances by pairwise comparisons (Figures 1 and 3; Table 1). However, an increased level of LD among Z-chromosomal markers was observed only among males (Li and Merilä, unpublished data). In the combined sample of both sexes, the reduced LD on the Z is in marked contrast to typical observations of higher LD on the X chromosome in mammals as in the case of humans (for example, Altshuler *et al.*, 2005), cattle (for example, Sandor *et al.*, 2006), and wild mice (for example, Laurie *et al.*, 2007). However, the findings would be compatible with theoretical expectations for differential selection on sex-linked loci under male and female heterogamy (Ellegren, 2009), as well as with the demonstration that the Z-chromosome is likely to be particularly sensitive to sexual selection in female heterogamy systems as compared to the X chromosome in XX/XY systems (see Reeve and Pfennig, 2003; Backström *et al.*, 2006). Another potential source for the reduction in LD on the Z-chromosome may concern the three pseudoautosomal markers (Jaari *et al.*, 2009), which constitute a large part of the Siberian jay LGZ. Recombination rates between markers in pseudoautosomal region are extremely high as compared with genomic averages because of obligate chiasma formation in this short region in every meiosis in the heterogametic sex as supported by mammalian studies (for example, Huang *et al.*, 2005). However, earlier studies in mammals included only or mostly X-specific markers, which in general show higher levels of LD than the pseudoautosomal markers (see Marshall *et al.*, 2004; Sandor *et al.*, 2006).

LD among nonsyntenic markers and prospects for LD mapping

The high level of LD is of great interest for future mapping studies of the Siberian jays, as it means that large quantitative trait regions rather than QTLs can be detected. Another striking feature of our results was the high proportion of nonsyntenic marker pairs in significant LD (Supplementary Figure S4). Earlier studies of LD have observed a mean nonsyntenic LD of 10–40% in domestic livestock (Farnir *et al.*, 2000; McRae *et al.*, 2002). However, 69–77% of nonsyntenic marker pairs were found to be in LD in a natural population of red deer (Slate and Pemberton, 2007), in which the pattern was ascribed to a recent admixture event. In this study, a potential explanation for the strong background LD is in the significant intrapopulation genetic structuring and the relatedness (c.f. pedigree samples) among individuals. Other potential explanations include small effective population size (c.f. Nsengimana *et al.*, 2004) of the study population ($N_e = 170$; Henna Fabritius, unpublished MSc thesis), as well as the sampling of related individuals in the pedigree. Whatever the explanation, the common occurrence of LD among nonsyntenic loci evokes serious concerns about the generation of type I errors when using LD mapping as the only means to locate genes underlying complex traits in the population (Farnir *et al.*, 2000). Therefore, a new strategy of joint linkage and LD mapping in natural populations (Wu and Zeng, 2001), which has the power to simultaneously capture the information about the linkage of the markers (as measured by recombination rate) and the degree of historical LD, would be preferred in a situation like this. Likewise, the considerable LD over long distances reduces precision in association-mapping context (Pritchard and Przeworski, 2001). A potential solution to this problem is to map QTLs in several populations simultaneously as the successful validation of QTLs depends on the sign of the association in different populations rather than on the strength of the initial association or its size of effect (Barendse *et al.*, 2007).

Molecular markers and LD metrics—some additional considerations

Although biallelic SNP markers are likely to predominate in LD-mapping studies of human populations, multiallelic microsatellites will continue to be useful in LD-mapping studies of livestock and wild vertebrates (for example, Farnir *et al.*, 2000; Slate and Pemberton, 2007; Lipkin *et al.*, 2009). The values obtained here for both D' and χ^2 at the <20 cM range are much greater than those reported for two other wild passerines (the collared flycatcher *Ficedula albicollis*, Backström *et al.*, 2006; the zebra finch *T. guttata*, Balakrishnan and Edwards, 2009) for biallelic SNP markers, using D' and the comparable r^2 measure. Thus, LD between multiallelic markers may indeed be greater and extend over longer distances than LD among biallelic SNP markers, which was also found in the LD studies in humans (Pritchard and Przeworski, 2001; Varilo *et al.*, 2003) and cattle (Khatkar *et al.*, 2006a,b). In addition, the relatively high rate of mutation in microsatellite markers makes possible the assessment of recently created LD

(Mountain *et al.*, 2002). Thus, microsatellite markers will continue to be useful and informative for studies of LD in the wild.

Currently there is no consensus with respect to the relative merits of the different measures (for example, D' , χ^2 , and r^2) of LD (but see Hudson, 2001). The metrics may have different context-dependent strengths. Slate and Pemberton (2007) recommended reporting both D' and χ^2 in the future studies to allow comparison of LD statistics across studies. As suggested, we calculated both D' and χ^2 metrics and chose to use D' in the further detailed analyses because the good biological and statistical properties of χ^2 may be outweighed by its frequency dependence and variable rate of decay (for example, Hedrick, 1987; Nsengimana *et al.*, 2004). Furthermore, use of D' facilitates the comparison of our results with those of wild vertebrate populations (for example, collared flycatcher, Backström *et al.*, 2006; red deer, Slate and Pemberton, 2007; and zebra finch, Balakrishnan and Edwards, 2009). In particular, when comparing samples with different allelic frequencies or different pairs of loci a frequency-independent measure such as D' is quite important (Hedrick, 1987). Moreover, D' is the measure of choice to assess recombination patterns as LD blocks have often been defined on the basis of D' (Chen *et al.*, 2006).

Conclusions

Knowledge about LD is important in the context of performing genome-wide association analysis and understanding strength and patterns of genomic selection (for example, Meadows *et al.*, 2008). The high levels of LD observed here suggest good prospects for association studies in this population, although ways to minimize the generation of false-positive results would need to be adopted. The patterns observed in this study also provide insights into the role of genomic selection in shaping, for example, patterns of genome variability and LD on the avian Z-chromosome. Furthermore, the findings presented here, together with those of a few other studies (Edwards and Dillon, 2004; Backström *et al.*, 2006; Balakrishnan and Edwards, 2009), indicate that wild birds could provide useful models for identifying genes underlying variation in complex traits of ecological and evolutionary interest.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by grants from the Academy of Finland, University of Helsinki, and Ministry of Education. We thank Bo-Göran Lilland for collection of the primary data. Thanks are also due to all individuals and funding bodies, which have—directly or indirectly—contributed to collection of the data. We are grateful to Sonja Jaari, Kaisa Välimäki for her help in the molecular data preparation, and Marika Karjalainen for technical assistance in laboratory. Niclas Backström, John Loehr, and four anonymous referees kindly and constructively commented on earlier versions of this paper.

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Supplementary Information accompanies the paper on Heredity website (<http://www.nature.com/hdy>)