doi:10.1111/j.1420-9101.2004.00694.x

Parasitism, host immune defence and dispersal

A. P. MØLLER,* M. MARTÍN-VIVALDI† & J. J. SOLER†

*Laboratoire de Parasitologie Evolutive, CNRS UMR 7103, Université Pierre et Marie Curie, Paris Cedex, France
†Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones Científicas, Almería, Spain

Keywords:

breeding dispersal;
cell-mediated immunity;
ecological plasticity;
natal dispersal;
phytohaemagglutinin.

Abstract

Host-parasite interactions have been hypothesized to affect the evolution of dispersal by providing a possibility for hosts to escape debilitating parasites, and by influencing the level of local adaptation. We used a comparative approach to investigate the relationship between a component of host immune function (which reflects the evolutionary history of parasite-induced natural selection) and dispersal in birds. We used a sample of 46 species of birds for which we had obtained field estimates of T-cell response for nestlings, mainly from our own field studies in Denmark and Spain. Bird species with longer natal, but not with longer breeding dispersal distances had a stronger mean T-cell-mediated immune response in nestlings than species with short dispersal distances. That was also the case when controlling for the potentially confounding effect of migration from breeding to wintering area, which is known from previous studies to be positively associated with dispersal distance. These relationships held even when controlling for similarity among species because of common ancestry. Avian hosts with a larger number of different breeding habitats had weaker mean T-cell-mediated immune responses than habitat specialists. This relationship held even when controlling for similarity among species because of common ancestry. Therefore, T-cell-mediated immunity is an important predictor of evolutionary changes in dispersal ability among common European birds.

Introduction

Dispersal is a life history trait with important consequences for ecology and evolution of populations (Clobert *et al.*, 2001). Dispersal will obviously affect the way in which individuals are distributed in space, which in turn will affect gene flow and genetic population structure. Ecological plasticity has been suggested to be associated with dispersal and the ability to locate new environments (Rosenzweig, 1975; Owens *et al.*, 1999), while others have suggested that ecological plasticity may restrict dispersal (Murren *et al.*, 2001).

Dispersal abilities will not only affect the number and identity of conspecifics with which an individual interacts, but also the intensity of such interactions, if individuals are not randomly distributed in space. Multiple causes for the evolution of dispersal have been proposed, including kin selection and kin competition (Gandon & Michalakis, 2001). Dispersal will also affect interactions with other species such as predators and parasites, and such interactions may have profound effects on the evolution of dispersal (reviews Boulinier *et al.*, 2001; Van Baalen & Hochberg, 2001). Here we investigate the prediction that the evolution of long dispersal distances are associated with large impact of parasites on their hosts and hence on the evolution of strong immune responses.

Ecological studies of the highly colonial cliff swallow *Petrochelidon pyrrhonota* have shown that local levels of ectoparasitism in breeding colonies affect natal dispersal distances, with fledglings dispersing further when intensities of local parasitism are high (Brown & Brown, 1992). Similarly, colonially breeding kittiwakes *Rissa tridactyla* infested with ticks of the species *Ixodes uriae* have higher breeding dispersal, but not natal dispersal,

Correspondence: Anders Pape Møller, Laboratoire de Parasitologie Evolutive, CNRS UMR 7103, Université Pierre et Marie Curie, 7 quai St Bernard, Case 237, F-75252 Paris Cedex 05, France. Tel.: (+33) 1 44 27 25 94; fax: (+33) 1 44 27 35 16; e-mail: amoller@snv.jussieu.fr

when the prevalence of the breeding cliffs is high (Boulinier *et al*., 2001). In less social hosts, Sorci *et al.* (1994) found that offspring dispersal of the common lizard *Lacerta vivipara* depended on tick infestation of their mothers. Studies of great tit *Parus major* nests experimentally infested with hen fleas *Ceratophyllus gallinae* showed that male offspring were more likely to recruit locally than females, when raised in infested nests (Heeb *et al*., 1999). A tendency for greater dispersal distances of yellow-bellied marmots *Marmota flaviventris* infested with ectoparasites also suggested that parasites affect dispersal (Van Vuren, 1996). In contrast, *Drosophila* infected with nematodes was not related to dispersal under field conditions, despite the fact that parasites reduced host survival (Jaenike *et al*., 1995).

Long dispersal distances may have evolved in situations where local parasite impact on reproductive success is severe (Møller & Erritzøe, 2001). At the interspecific level we might predict a positive association between dispersal distance and immune response, as a direct link between parasite-induced mortality and host level of immune defence exists (Martin *et al*., 2001). Several potential examples exist of hosts moving out of the range of parasites (Freeland, 1980; Folstad *et al*., 1991), resulting in a decrease of parasite impact on host fitness.

The level of local adaptation in host–parasite systems has been hypothesized to depend on the relative rate of migration of hosts and parasites (Gandon *et al*., 1996; Kaltz & Shykoff, 1998). Based on the meta-population model of Gandon *et al.* (1996), host populations are expected to be locally adapted when parasites migrate more than hosts, while local mal-adaptation should occur when hosts migrate more than parasites. These predictions are supported by some empirical studies (Kaltz & Shykoff, 1998). However, migration rates of hosts may change in response to the presence of parasites, making the interacting parties change their relative benefits. For example, a study of interactions between the brood parasitic great spotted cuckoo *Clamator glandarius* and its magpie *Pica pica* host revealed little difference in migration rate between host and parasite in sympatry, while allopatric host populations had considerably smaller migration rates than sympatric host populations (Martínez *et al*., 1999). This suggests that host migration rate may change in response to the presence of parasites. Thus, long genetic migration distances by hosts (which may equate with long dispersal distances) are expected to provide hosts with a selective advantage in their coevolutionary interactions with parasites. Hence, we might predict that long dispersal distances should be associated with stronger immune responses of individual hosts.

The degree of ecological plasticity may affect host–parasite interactions because specialization by parasites on particular host species is more likely when the host is restricted to one or a few habitats (Tripet *et al*., 2002). Superficially, this might occur because only a few parasite species are encountered. However, that is not the case as many colonially breeding avian hosts in fact encounter more species of parasites than solitary species because mixed colonies of different host species with different specialist parasites commonly breed in the same colony (Tripet *et al*., 2002). As habitat specialization is associated with parasites specializing on particular hosts, a direct link between ecological plasticity and immune response can be predicted at the interspecific level. The sign of this hypothetical relationship would depend on the relative selection pressures exerted by parasites on hosts varying in ecological plasticity. A small range of habitats will increase the intensity of interactions between hosts and parasites, and may therefore result in specialization and speciation by the parasite counteracting host defences. Specific hosts should increase their defences against parasites because increases in host defence will select for increases in offence by the parasite. Therefore, a negative relationship between ecological plasticity and immune response is predicted at the interspecific level. A habitat generalist host is likely to encounter many different kinds of potential generalist parasites, and the rate of horizontal transmission of parasites among different species of hosts may therefore be larger in generalist than in habitat specialist hosts. Generalist parasites may be restricted to host species with relatively low levels of immune defence, because they are unable to cope with the strong defences of specialists. The coevolutionary process between parasites and hosts may be relatively slow when several hosts and parasites are involved (diffuse coevolution) in comparison to cases when only a couple of species are involved (strict coevolution) (Thompson, 1994). As it is likely that selection pressures on hosts exerted by specialist parasites are stronger than those exerted by generalist parasites, and habitat specialist hosts are likely to suffer more from specialist parasites than habitat generalist hosts, the relationship between ecological plasticity as reflected by the number of habitats and immune response should be negative.

The aims of this study were to assess the relationships between dispersal distance and immune function, using birds as a model system. Specifically, we investigated the interspecific relationship between dispersal distance and a component of immune response, after taking a number of potentially confounding variables into account. Information on dispersal distances for 75 bird species was reported by Paradis *et al.* (1998). As a measure of immune response, we used a standard measure of T-cell-mediated immune response to subcutaneous challenge of nestlings at a standardized age with a novel antigen (phytohaemagglutinin). A previous comparative study has shown that bird species that suffer from intense parasite-induced nestling mortality indeed have evolved stronger cell-mediated immune responses than species with little or no mortality (Martin *et al*., 2001). We included a measure of ecological plasticity estimated as

the number of breeding habitats used by each species, according to a standard handbook on Western Palaearctic birds. Furthermore, previous studies have shown that migratory bird species have longer dispersal distances than resident birds (Paradis *et al.*, 1998; Belliure *et al.*, 2000). Hence, a measure of migration distance was included in the analyses. Finally, hole nesting and colonially breeding birds encounter parasites that induce higher degrees of nestling mortality than open nesting and solitarily breeding birds (Møller & Erritzøe, 1996; Møller *et al.*, 2001). As a consequence, the former categories of species have evolved stronger immune responses than the latter species (Møller & Erritzøe, 1996; Møller *et al.*, 2001). As hole nesting and coloniality may restrict the ecological plasticity of a species, we controlled for these variables when analysing the relationship between immune function and ecological plasticity.

Material and methods

Dispersal distance

We used dispersal distances of the bird species breeding in Britain, as reported by Paradis *et al.* (1998). This data set contains information on mean dispersal distances to the nearest kilometre, as recorded by the British Trust for Ornithology during an extended period 1909–1994. Natal dispersal was defined as the distance moved by individuals ringed in their year of hatching and recovered at breeding age, while breeding dispersal was defined as the distance moved by individuals ringed at breeding age. Detailed information about data compilation and calculations is provided by Paradis *et al.* (1998). We used the geometric mean dispersal distances in the present analyses.

T-cell-mediated immunity

We included all species for which information on T-cell-mediated immune response had been published by 31 July 2001 (three species *C. glandarius, Luscinia svecica, Serinus serinus* ; Soler *et al.*, 1999; Johnsen *et al.*, 2000; Hoi-Leitner *et al.*, 2001), or for which we or our collaborators had recorded responses to the phytohaemagglutinin test (the remaining species). Only the tests on *S. serinus* (0.5 mg phytohaemagglutinin in 0.10 mL physiological water) and *L. svecica* (0.1 mg in 0.04 mL physiological water) were not performed in the same way as the test for the remaining species, but exclusion of these species did not change the findings for number of breeding habitats (there was no information on dispersal distance for these species). During the breeding seasons 2000–2001 we spent large parts of April to June searching for nests of birds, in which nestlings could be tested for cell-mediated immune response. This was done in Southern Spain around Granada and Sierra Nevada in 2000 and in Northern Denmark in 2000–2001. We requested information on nests from a number of amateur ornithologists with a good knowledge of birds (see Acknowledgments), and the nests recorded in this way were monitored until the test was being performed. As a measure of immune response we used the T-cell-mediated immune response to a challenge with phytohaemagglutinin. This is a standard estimate from the poultry literature of the ability to produce a T-cell-mediated immune response (Goto *et al.*, 1978; McCorkle *et al.*, 1980; Parmentier *et al.*, 1993; Dietert *et al.*, 1996). Injection with phytohaemagglutinin results in local activation and proliferation of T-cells, followed by local recruitment of inflammatory cells and increased expression of major histocompatibility complex molecules (Goto *et al.*, 1978; Abbas *et al.*, 1994; Parmentier *et al.*, 1998). Before injection we removed the feathers from a small spot of skin on the wing web (patagium) of the right and the left wings and marked the sites of injection with a permanent, water-resistant colour marker. We then measured the thickness of the skin to the nearest 0.01 mm with a pressure-sensitive calliper (Teclock SM112, Japan). For each wing web we made three measurements to quantify measurement error. As in previous studies we found highly repeatable measurements, with repeatabilities above 0.95. This is similar to our previous studies of T-cell-mediated immunity (e. g. Saino *et al.*, 1997; Soler *et al.*, 1999; Martin *et al.*, 2001; Merino *et al.*, 2001). Subsequently, we injected 0.02 mg phytohaemagglutinin dissolved in 0.04 mL physiological water in one wing web, and 0.04 mL physiological water in the other wing web. Approximately 24 h later we remeasured the thickness of the skin at the two sites of injection, as described above. The index of cell-mediated immune response was simply calculated as the difference in thickness of the wing web injected with phytohaemagglutinin 24 h after and just before injection, minus the difference in thickness of the wing web injected with physiological water. Thus, the measure of response is expressed in millimetre. We calculated mean responses for each brood and then calculated an overall mean based on these brood mean values. The data set is presented in the Appendix.

We have found highly significant, consistent differences in cell-mediated immune response in nestlings among species when testing these differences with a one-way ANOVA (calculations based on residual T-cell response from a regression of log-transformed T-cell response on log-transformed body mass: $F_{65,604} = 6.375$, SS (species) = 35.780, SS (residual) = 65.192, $P < 0.001$, data from Denmark 2001). This implies that there is considerably variation among species in relative immune response. Furthermore, we have found for 18 species with mean estimates available from both Spain and Denmark that there is significantly more variation among than within species in mean cell-mediated immune response (one-way ANOVA based on residual T-cell response from a regression of log-transformed T-cell

response on log-transformed body mass: $F_{17,18} = 7.675$, SS (species) = 1.623, SS (residual) = 0.224, $P < 0.001$). Nestlings were injected at a standard relative age during their ontogeny (when they were two thirds through their normal nestling period) rather than at a similar absolute age. This procedure ensured that nestlings were tested at a similar developmental stage, approximately equalling break of wing feathers through the feather pins, which reduces the effects of recorded responses being dependent on developmental age. Preliminary studies of age-dependent cell-mediated immunity in barn swallow *Hirundo rustica* nestlings have shown little variation in intensity of response during the period 10–16 days (in a species with a 20 days nestling period) (A. P. Møller unpublished data).

Although a thorough characterization of immunocompetence (the ability to raise an efficient response to a parasite attack) requires that T- and B-cell mediated and innate immunity are quantified (National Research Council, 1992), we suggest that a single measure recorded in a standardized across a range of species is superior to no measure at all. Furthermore, we note that at the interspecific level T-cell-mediated immune response is positively correlated with antibody production to a challenge with sheep red blood cells across a sample of 13 species of hirundines (Møller *et al*., 2001). Thus, two components of immunocompetence covary positively across species of birds.

Ecological plasticity

We recorded the number of breeding habitats by using the habitat preferences that Cramp & Simmons (1977–1994) reported for all species. These authors define the principal habitat categories in a glossary, and only use these for characterization of the habitat use of the species. We enumerated these habitat categories in the breeding habitat descriptions of each species. The index used was simply the total number of habitat categories reported. As our study was made independently of these habitat preferences published by Cramp and Simmons, we can assume that we have not introduced bias in the classification.

Ecological variables

Migratory bird species are known to disperse further than resident bird species (Paradis *et al*., 1998; Belliure *et al*., 2000). Hence, we quantified migration distance as the distance between the centre of the breeding distribution and the centre of the winter distribution. The centre was simply calculated as the mean value of the northernmost and the southernmost latitude of the breeding and the wintering distributions, respectively. This measure of distribution has previously been shown to provide a biologically meaningful measure (Gaston & Blackburn, 1996). The difference between these two mean latitudes was considered to represent the migration distance. These distances were recorded to the nearest 500 km on a map. Information on breeding and wintering ranges was obtained from Cramp & Simmons (1977–1994).

Bird species with hole nests suffer more from the effects of parasites than open nesting species, and they have stronger immune responses as shown by their larger relative size of bursa of Fabricius and spleen (Møller & Erritzøe, 1996). Likewise, hole nesters have more specific habitat requirements than open nesters, which can place their nests in many more different sites than hole nesters (Lack, 1968), and this may affect their ecological plasticity. Nest sites were classified as hole nests, when the nest is placed in a cavity, crevice or a similar structure, while all other species were classified as open nesters. Information was obtained from Cramp & Simmons (1977–1994).

Colonially breeding bird species suffer more from the effects of parasites than solitarily nesting species (Møller *et al*., 2001), and they have stronger immune responses as shown by their larger relative size of bursa of Fabricius and spleen and stronger T- and B-cell responses (Møller & Erritzøe, 1996; Møller *et al*., 2001). Species were classified as colonially nesting if more than a single pair regularly breed aggregated in space, while all other species were classified as solitarily nesting. likewise, colonial species breed in sites with specific requirements with respect to safety from predators and proximity to suitable foraging sites (Lack, 1968), and this may affect their ecological flexibility. Colony size was recorded on a logarithmic scale with maximum colony sizes of 10, 100, 1000 10 000 and 100 000 being scored as 1, 2, 3, 4 and 5. Information was obtained from Cramp & Simmons (1977–1994).

We used information on body mass from Dunning (1993). All data are reported in the Appendix.

Statistical methods

Information on natal and breeding dispersal was not available for all species, and sample sizes therefore differ among analyses. Mean geometric dispersal distances, cell-mediated immune response and adult body mass were $\log_{10}$-transformed before analysis to achieve distributions that did not differ from normal distributions. As we have found a consistent intraspecific difference in immune response between Denmark and Spain, we included a dummy variable in the preliminary analyses, by assigning a value of 0.0 to a mean immune response from Spain, a value of 0.5 to a mean immune response from Spain and Denmark, and a value of 1.0 to a mean immune response from Denmark. Inclusion of this variable did not change any of the conclusions presented in the results. Hence, our findings were robust with respect to the geographical origin of the individual data points.

Allometry effects of cell-mediated immune responses were controlled by using log-transformed body mass as a covariate in the analyses. Across species we found a

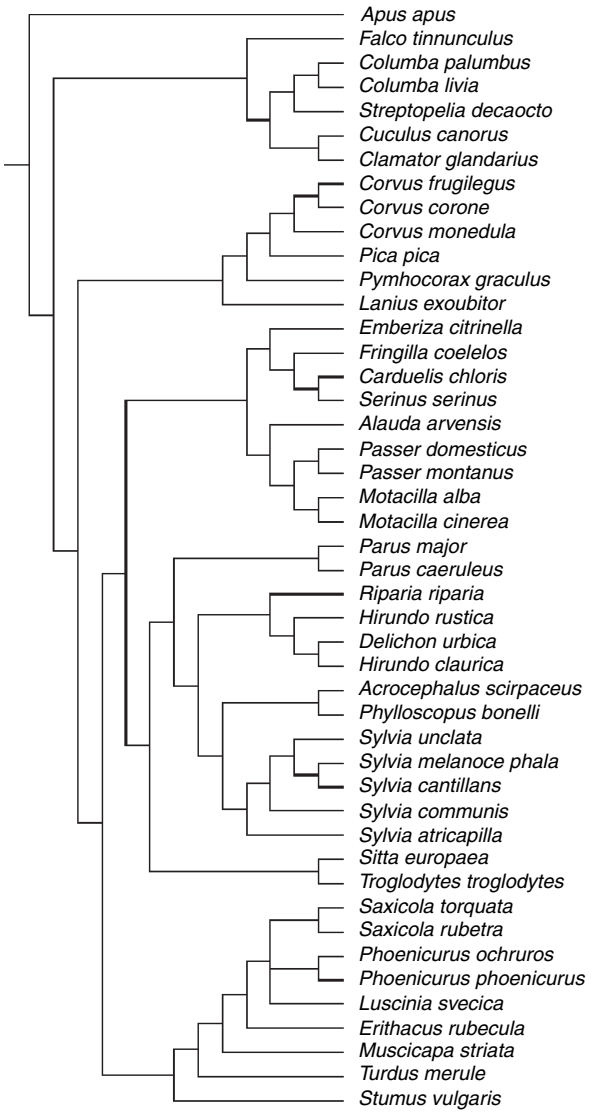

Fig. 1 Composite phylogeny used for the comparative analyses. See *Material and methods* for sources.

significant positive relationship between $\log_{10}$-transformed immune response and $\log_{10}$-transformed body mass [$F_{1,64} = 20.31$, $r^2 = 0.241$, $P < 0.001$, slope (SE) = 0.228 (0.051)]. However, a similar regression between $\log_{10}$-transformed immune response and $\log_{10}$-transformed skin thickness before injection was not significant [$F_{1,64} = 2.25$, $r^2 = 0.051$, $P = 0.08$, slope (SE) = 0.202 (0.134)]. A multiple linear regression with $\log_{10}$-transformed immune response as the dependent variable and $\log_{10}$-transformed body mass and $\log_{10}$-transformed skin thickness before injection as independent variables only revealed a significant partial regression coefficient for body mass, but not for skin thickness [$F_{2,63} = 11.14$, $r^2 = 0.352$, $P < 0.0001$, slope (SE) for body mass = 0.347 (0.079), $t = 4.37$, $P < 0.0001$; slope (SE) for skin thickness = 0.043 (0.118), $t = 0.37$, $P = 0.716$]. Use of $\log_{10}$-transformed body mass thus corrects efficiently for interspecific differences in body size without causing any bias due to initial thickness of skin among species.

Phenotypic mean values for species cannot be considered statistically independent observations because cases of convergent evolution are mixed with cases of similarity because of common ancestry. We calculated statistically independent linear contrasts for each variable according to the method developed by Felsenstein (1985). We used a composite phylogeny based on the phylogenies in Sibley & Ahlquist (1990), Sheldon & Winkler (1993), Blondel *et al.* (1996), Martin & Clobert (1996) and Leisler *et al.* (1997). The phylogeny is shown in Fig. 1. We adopted the software CAIC to make the calculations of contrasts (Purvis & Rambaut, 1995). All branches were assigned the same length, although a second set of analyses based on uneven branch lengths, assuming a gradual evolution model as implemented in the software by Purvis & Rambaut (1995), produced qualitatively similar results. We tested for violations of statistical assumptions by regressing standardized contrasts against their standard deviations (Garland *et al.*, 1992). None of these tests revealed any significant deviations, after Bonferroni adjustment for multiple tests. Contrasts were analysed by forcing regressions through the origin (Purvis & Rambaut, 1995). Multiple regression models and forward stepwise multiple regression models were used to control for the effects of confounding variables.

Sample sizes differ among tests because information on dispersal distance was only available for a sub-sample of the species.

Results

Natal and breeding dispersal distance were not significantly related to cell-mediated immune response in a multiple regression with $\log_{10}$-transformed body mass as an additional independent variable, when using species as independent observations [Fig. 2a, b; multiple linear regression: natal dispersal: $F_{2,28} = 1.41$, $r^2 = 0.091$, $P = 0.261$, slope (SE) = 0.752 (0.454), $t = 1.657$, $P = 0.109$; breeding dispersal: $F_{2,26} = 0.28$, $r^2 = 0.021$, $P = 0.759$, slope (SE) = 0.295 (0.407), $t = 0.726$, $P = 0.474$]. That was also the case when basing the calculations on contrasts [multiple linear regression: natal dispersal: $F_{2,27} = 2.90$, $r^2 = 0.172$, $P = 0.072$, slope (SE) = 0.180 (0.082), $t = 2.198$, $P = 0.036$; breeding dispersal: $F_{2,25} = 0.72$, $r^2 = 0.052$, $P = 0.498$, slope (SE) = 0.074 (0.122), $t = 0.605$, $P = 0.550$].

As migratory birds disperse longer than resident birds (Paradis *et al.*, 1998; Belliure *et al.*, 2000), we used migration distance as a third independent variable. Dispersal and migration distance was not significantly

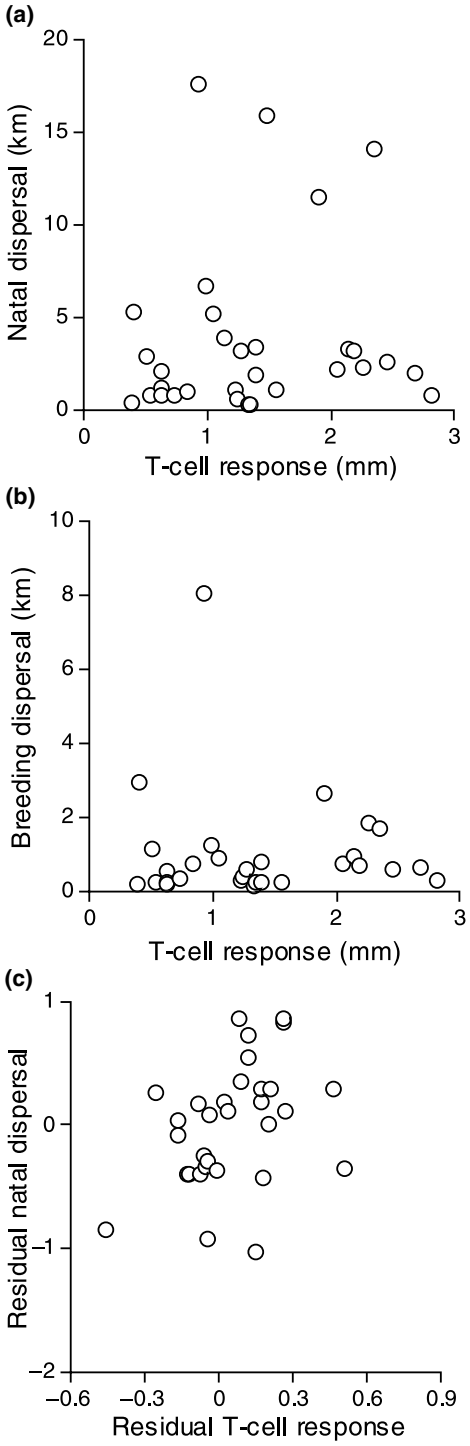

Fig. 2 Dispersal distance in relation to T-cell-mediated immune response of nestling birds. (a) Natal dispersal distance (km). (b) Breeding dispersal distance (km). (c) Residual natal dispersal distance after controlling for number of breeding habitats and $\log_{10}$-transformed body mass in a multiple regression analysis and residual T-cell response after controlling for the effect of $\log_{10}$-transformed body mass.

Table 1 Multiple regression analyses with T-cell-mediated immune response as the dependent variable and natal dispersal distance, breeding dispersal distance, migration distance and body mass as independent variables.

Source	Slope (SE)	*t*	*P*
Species as observations			
Model: $F = 4.62$, d.f. = 4,24, $r^2 = 0.435$, $P = 0.0066$			
Natal dispersal	0.172 (0.156)	1.107	0.279
Breeding dispersal	−0.145 (0.180)	0.805	0.429
Migration	0.039 (0.065)	0.603	0.552
Body mass	0.317 (0.086)	3.690	0.0011
Contrasts as observations			
Model: $F = 1.05$, d.f. = 4,24, $r^2 = 0.149$, $P = 0.403$			
Natal dispersal	0.211 (0.128)	1.648	0.112
Breeding dispersal	−0.072 (0.150)	0.480	0.635
Migration	−0.026 (0.102)	0.256	0.801
Body mass	0.192 (0.174)	1.106	0.280

Table 2 Multiple regression analyses with T-cell-mediated immune response as the dependent variable and number of breeding habitats, natal dispersal distance, migration distance, hole nesting, coloniality and body mass as independent variables.

Source	Slope (SE)	*t*	*P*
Species as observations			
Model: $F = 7.32$, d.f. = 6,24, $r^2 = 0.647$, $P = 0.0002$			
No. breeding habitats	−0.020 (0.012)	1.743	0.094
Natal dispersal	0.103 (0.078)	2.085	0.048
Migration	−0.027 (0.055)	0.492	0.628
Hole nesting	0.026 (0.073)	0.363	0.719
Coloniality	0.136 (0.094)	1.444	0.162
Body mass	0.236 (0.077)	3.064	0.0053
Contrasts as observations			
Model: $F = 6.06$, d.f. = 6,24, $r^2 = 0.603$, $P = 0.0006$			
No. breeding habitats	−0.029 (0.008)	3.418	0.0023
Natal dispersal	0.235 (0.077)	3.069	0.0053
Migration	−0.021 (0.062)	0.331	0.744
Hole nesting	−0.055 (0.067)	0.825	0.418
Coloniality	0.096 (0.084)	1.151	0.261
Body mass	0.214 (0.122)	1.760	0.091

related to cell-mediated immunity (Table 1). A similar conclusion was reached by a forward stepwise linear regression analysis.

Cell-mediated immunity was smaller is bird species with a larger number of breeding habitats. When controlling for similarity because of common ancestry by using contrasts as independent observations we found a highly significant negative relationship between cell-mediated immunity and number of breeding habitats (Table 2). That was also the case when controlling statistically for the potentially confounding effect of hole nesting and coloniality on number of breeding habitats (Table 2). However, in the complete data set including species without information

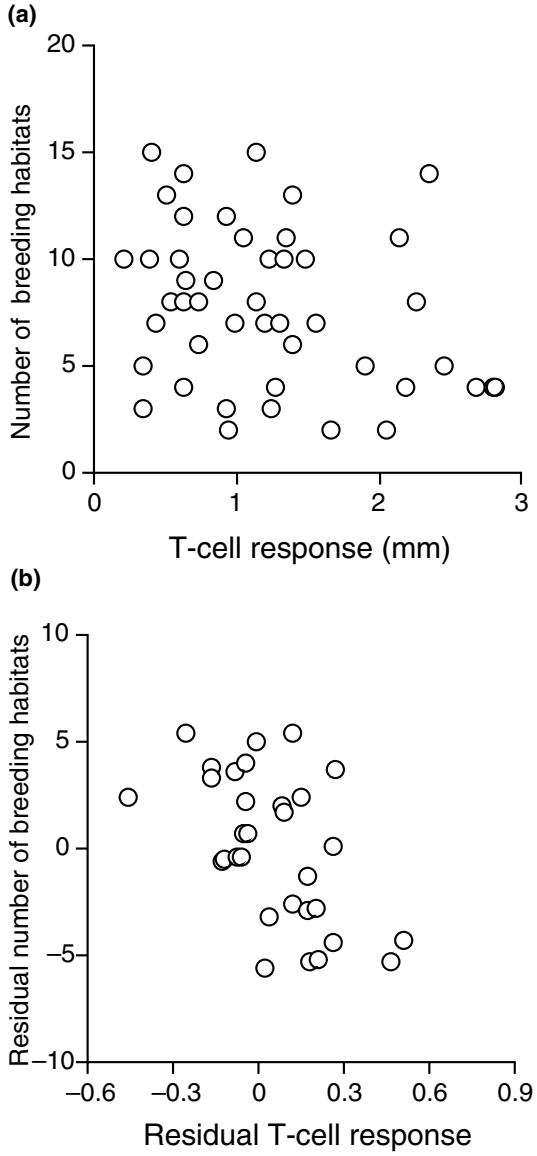

Fig. 3 T-cell-mediated immune response of nestlings (mm) in relation to number of breeding habitats in birds. (a) Number of breeding habitats. (b) Residual number of breeding habitats after controlling for the effect of natal dispersal distance and $\log_{10}$-transformed body mass in a multiple regression analysis and residual T-cell response after controlling for the effect of $\log_{10}$-transformed body mass.

on dispersal distance there was no such relationship [Fig. 3a; multiple linear regression including body mass as an additional independent variable: $F_{2,43} = 20.02$, $r^2 = 0.482$, $P < 0.001$, partial regression coefficient for number of breeding habitats: slope (SE) = −0.015 (0.008), $t = 1.801$, $P = 0.079$; analysis based on contrasts ($F_{2,41} = 1.68$, $r^2 = 0.076$, $P = 0.199$, partial regression coefficient for number of breeding habitats: slope (SE) = −4.577 (3.312), $t = 1.382$, $P = 0.175$]. Thus, it was only when entering dispersal distance as a covariate that the relationship between number of breeding habitats and cell-mediated immunity became significant.

When cell-mediated immunity of the different species was entered as the dependent variable and both natal dispersal distance and number of breeding habitats as independent variables, immune response was significantly positively related to natal dispersal distance (Fig. 2c) and significantly negatively related to the number of breeding habitats (Fig. 3b; Table 2). That was even the case when using contrasts as independent observations (Table 2). Breeding dispersal distance was not significantly related to cell-mediated immunity when added as an independent variable to the multiple regression in Table 2 [$F_{7,21} = 4.34$, $r^2 = 0.592$, $P = 0.199$, partial regression coefficient: slope (SE) = 0.010 (0.117), $t = 0.084$, $P = 0.934$], while the partial regression coefficients for natal dispersal and number of habitats remained statistically significant.

Discussion

The main findings of the present study were that interspecific variation in a measure of T-cell-mediated immune response was positively correlated with variation in natal dispersal distance, but not with breeding dispersal distance. Furthermore, cell-mediated immune response was negatively correlated with the number of breeding habitats. Further analyses showed that they were independent of any relationship between migration and dispersal, and any relationship between ecological specialization and hole nesting or colonial breeding. The magnitude of the effect size measured in terms of Pearson's correlation coefficient [where $r^2 = t^2/(t^2 + \text{d.f.})$ (Rosenthal, 1994)] was 28.2% of the interspecific variance in T-cell response explained by natal dispersal and 32.7% of the variance explained by ecological plasticity (data from Table 2). Effects are considered by Cohen (1984) to be strong, when the amount of variance explained is 25%. As all these analyses were correlational in nature, we do not invoke any causation in the relationships discussed here. Obviously, our measure of T-cell-mediated immune response is only one component of immunocompetence (National Research Council, 1992), but our approach should be feasible even for other components, although these will be more difficult to obtain for a large number of species under field conditions.

Natal dispersal distance is positively related to breeding dispersal distance across species ($r = 0.781$; Paradis *et al.*, 1998; Belliure *et al.*, 2000), although breeding dispersal is much shorter than natal dispersal. We found a positive relationship between natal dispersal distance and cell-mediated immunity (Fig. 2c), while a similar relationship was absent for breeding dispersal (Fig. 2b). Birds may develop adaptive immune responses to the parasite fauna

of their breeding sites (and migration and wintering sites), and such phenotypic adaptation may be particularly prominent among juvenile and prebreeding life stages (Møller & Erritzøe, 2001). Once established in a breeding site, with well-developed immune responses to local genotypes of parasites, any further long-distance movement may be detrimental because of age-specific 'vaccination' and antibody production towards local parasite strains (Møller & Erritzøe, 2001). The positive relationship between cell-mediated immunity and natal dispersal distance, but not between cell-mediated immunity and breeding dispersal distance, is therefore expected.

It may seem surprising that we find so clear results, given that dispersal distances were recorded in Britain, breeding habitat preferences throughout the distributional range of the species, and cell-mediated immune responses mainly were recorded in Denmark and Spain. However, Belliure *et al.* (2000) have previously shown that natal dispersal distance is a good predictor of relative subspecies richness in birds, implying that dispersal distances of birds from Britain are predictors of phenomena well beyond the British Isles. Furthermore, we have data showing strong geographical differences in phytohaemagglutinin response of the same species in Denmark and Spain, although analyses of variance clearly indicate consistency in response among populations of the same species (see Materials and methods). Thus, we have shown for dispersal distance and cell-mediated immune response that estimates for different species are reliable predictors of ecological and evolutionary relationships beyond the populations investigated.

Ecological generalists were found to have weaker T-cell-mediated immunity than habitat specialists (Fig. 3). This relationship held even when taking nest site and breeding aggregation into account as potentially confounding variables. We predicted this negative relationship because a larger number of habitats would allow escape from specialist parasites. Ecological flexibility has been proposed to reflect colonizing ability and the probability that a taxon will diversify (Rosenzweig, 1975; Owens *et al*., 1999). As short dispersal distances are associated with a high degree of subspeciation and hence of diversification (Belliure *et al*., 2000), and as bird species with short dispersal distances have weak cell-mediated immune responses (Fig. 2), we can infer that a large number of breeding habitats indeed seems to reflect the probability of a taxon diversifying.

Belliure *et al.* (2000) found no significant relationship between the number of breeding habitats and dispersal distance in birds, suggesting that long distance dispersers do not have more opportunities to colonize a novel habitat. However, as short dispersal distances are associated with a higher degree of sub-speciation and hence of diversification (Belliure *et al*., 2000), and as the impact of parasitism measured as the level of cell-mediated immune response predicts both dispersal distance and number of breeding habitats (see Results), we can hypothesize that parasites have a dampening effect on diversification within host species and therefore possibly on speciation among hosts. Although the mere existence of subspecies as a biological entity has been disputed (Mayr, 1942, 1963), morphological differentiation can be considered to represent initial steps in divergence among populations (Houde & Endler, 1990). Thus, parasitism through its effects on the evolution of dispersal distances may reduce the rate of speciation in hosts.

In conclusion, we have provided evidence for relationships between natal dispersal distance, breeding habitat specialization and a component of immune function in birds. These findings suggest that parasites have either directly or indirectly played a significant role in the evolution of dispersal distances in common species of European birds.

Acknowledgments

W. C. Aarestrup, Ph. Christe, E. Flensted-Jensen, M. Houmøller, K. Klarborg, J. M. Marín, P. Pap and T. Pérez kindly helped to search for nests and/or make phytohaemagglutinin tests. Their efforts were indispensable for the success of our study. J. Clobert and T. Martin provided constructive criticism. M. M. V. was supported by a grant from Fundación Caja de Madrid.

References

Abbas, A.K., Lichtman, A.H. & Pober, J.S. 1994. *Cellular and Molecular Immunology*. Saunders, Philadelphia.

Belliure, J., Sorci, G., Møller, A.P. & Clobert, J. 2000. Dispersal distances predict subspecies richness in birds. *J. Evol. Biol.* **13**: 480–487.

Blondel, J., Catzeflis, F. & Perret, P. 1996. Molecular phylogeny and the historical biogeography of the warblers of the genus *Sylvia* (Aves). *J. Evol. Biol.* **9**: 871–891.

Boulinier, T., McCoy, K. & Sorci, G. 2001. Dispersal and parasitism. In: *Dispersal* (J. Clobert, J.D. Nichols, E. Danchin & A. Dhondt, eds), pp. 169–179. Oxford University Press, Oxford, UK.

Brown, C.R. & Brown, M.B. 1992. Ectoparasitism as a cause of dispersal in cliff swallows. *Ecology* **73**: 1718–1723.

Clobert, J., Nichols, J.D., Danchin, E. & Dhondt, A. (eds) 2001. *Dispersal*. Oxford University Press, Oxford, UK.

Cohen, J. 1984. *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. L. Erlbaum, Hillsdale, NJ.

Cramp, S. & Simmons, K.E.L. (eds) 1977–1994. *The Birds of the Western Palearctic*, Vols. 1–10. Oxford University Press, Oxford, UK.

Dietert, R.R., Golemboski, K.A., Kwak, H., Ha, R. & Miller, T.E. 1996. Environment-immunity interactions. In: *Poultry Immunology* (T.F. Davison, T.R. Morris & L.N. Payne, eds), pp. 343–356. Carfax, Abingdon, UK.

Dunning, J.B. 1993. *CRC Handbook of Avian Body Masses*. CRC Press, Boca Raton, Florida.

Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**: 1–15.

Folstad, I., Nilssen, A., Halvorsen, A. & Andersen, J. 1991. Parasite-avoidance: the cause of post-calving migration in *Rangifer*? *Can. J. Zool.* **69**: 2423–2429.

Freeland, W.J. 1980. Mangabey (*Cercocebus albigena*) movement patterns in relation to food availability and fecal contamination. *Ecology* **61**: 1297–1303.

Gandon, S. & Michalakis, Y. 2001. Multiple causes of the evolution of dispersal. In: *Dispersal* (J. Clobert, J.D. Nichols, E. Danchin & A. Dhondt, eds), pp. 155–167. Oxford University Press, Oxford, UK.

Gandon, S., Capowiez, Y., Dubois, Y., Michalakis, Y. & Olivieri, I. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proc. R. Soc. Lond. B Biol. Sci.* **263**: 1003–1009.

Garland, Jr, T., Harvey, P.H. & Ives, A.R. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Am. Nat.* **41**: 18–32.

Gaston, K.J. & Blackburn, T.M. 1996. Global scale macroecology: interactions between population size, geographic range size and body size in the Anseriformes. *J. Anim. Ecol.* **65**: 701–714.

Goto, N., Kodama, H., Okada, K. & Fujimoto, Y. 1978. Suppression of phytohaemagglutinin skin response in thymectomized chickens. *Poult. Sci.* **52**: 246–250.

Heeb, P., Werner, I., Mateman, A.C., Kölliker, M., Brinkhof, M.W.G., Lessells, C.M. & Richner, H. 1999. Ectoparasite infestation and sex biased local recruitment of hosts. *Nature* **400**: 63–65.

Hoi-Leitner, M., Romero-Pujante, M., Hoi, H. & Pavlova, A. 2001. Food availability and immune capacity in serin (*Serinus serinus*) nestlings. *Behav. Ecol. Sociobiol.* **49**: 333–339.

Houde, A.E. & Endler, J.A. 1990. Correlated evolution of female mating preference and male color patterns in the guppy *Poecilia reticulata*. *Science* **248**: 1405–1408.

Jaenike, J., Benway, H. & Stevens, G. 1995. Parasite-induced mortality in mycophagous Drosophila. *Ecology* **76**: 383–391.

Johnsen, A., Andersen, V., Sunding, C. & Lifjeld, J.T. 2000. Female blue throats enhance offspring immunocompetence through extra-pair copulations. *Nature* **406**: 296–299.

Kaltz, O. & Shykoff, J. 1998. Local adaptation in host-parasite systems. *Heredity* **81**: 361–370.

Lack, D. 1968. *Ecological Adaptations for Breeding in Birds*. Methuen, London, UK.

Leisler, B., Heidrich, P., Schulze-Hagen, K. & Wink, W. 1997. Taxonomy and phylogeny of reed warblers (genus *Acrocephalus*) based on mtDNA sequences and morphology. *J. für Ornithologie* **138**: 469–496.

Møller, A.P. & Erritzøe, J. 1996. Parasite virulence and host immune defence: host immune response is related to nest re-use in birds. *Evolution* **50**: 2066–2072.

Møller, A.P. & Erritzøe, J. 2001. Dispersal, vaccination and regression of immune defence organs. *Ecol. Lett.* **4**: 484–490.

Møller, A.P., Merino, S., Brown, C.R. & Robertson, R.J. 2001. Immune defense and host sociality: a comparative study of swallows and martins. *Am. Nat.* **158**: 136–145.

McCorkle, F. Jr, Olah, I. & Glick, B. 1980. The morphology of the phytohemagglutinin-induced cell response in the chicken wattle. *Poult. Sci.* **59**: 616–623.

Martin, T.E. & Clobert, J. 1996. Nest predation and avian life history evolution in Europe versus North America: a possible role for humans? *Am. Nat.* **147**: 1028–1046.

Martin, T.E., Møller, A.P., Merino, S. & Clobert, J. 2001. Does clutch size evolve in response to parasites and immunocompetence? *Proc. Natl. Acad. Sci. U. S. A.* **98**: 2071–2076.

Martínez, J.G., Soler, J.J., Soler, M., Møller, A.P. & Burke, T. 1999. Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*), and its host, the magpie (*Pica pica*). *Evolution* **53**: 269–278.

Mayr, E. 1942. *Systematics and the Origin of Species*. Columbia University Press, New York, NY.

Mayr, E. 1963. *Animal Species and Evolution*. Harvard University Press, Cambridge, MA.

Merino, S., Martínez, J., Møller, A.P., Barbosa, A., de Lope, F. & Rodríguez-Caabeiro, F. 2001. Physiological and hematological consequences of a novel parasite on the red-rumped swallow *Hirundo daurica*. *Int. J. Parasitol.* **31**: 1187–1193.

Murren, C.J., Julliard, R., Schlichting, C.D. & Clobert, J. 2001. Dispersal, individual phenotype and phenotypic plasticity. In: *Dispersal* (J. Clobert, J.D. Nichols, E. Danchin & A. Dhondt, eds), pp. 261–272. Oxford University Press, Oxford, UK.

National Research Council 1992. *Biologic Markers in Immunotoxicology*. National Academy Press, Washington, DC.

Owens, I.P.F., Bennett, P.M. & Harvey, P.H. 1999. Species richness among birds: body size, life history, sexual selection or ecology? *Proc. R. Soc. Lond. B Biol. Sci.* **266**: 933–939.

Paradis, E., Baillie, S.R., Sutherland, W.J. & Gregory, R.D. 1998. Patterns of natal and breeding dispersal in birds. *J. Anim. Ecol.* **67**: 518–536.

Parmentier, H.K., Schrama, J.W., Meijer, F. & Nieuwland, M.G.B. 1993. Cutaneous hypersensitivity responses in chickens divergently selected for antibody responses to sheep red blood cells. *Poult. Sci.* **72**: 1679–1692.

Parmentier, H.K., de Vries Reilingh, G. & Nieuwland, M.G.B. 1998. Kinetic immunohistochemical characteristic of mitogen-induced cutaneous hypersensitivity responses in chickens divergently selected for antibody responsiveness. *Vet. Immunol. Immunopathol.* **66**: 367–376.

Purvis, A. & Rambaut, A. 1995. Comparative analysis by independent contrasts (CAIC). *Comp. Appl. Biosci.* **11**: 247–251.

Rosenthal, R. 1994. Parametric measures of effect size. In: *The Handbook of Research Synthesis* (H. Cooper & L.V. Hedges, eds), pp. 231–244. Russell Sage Foundation, New York, NY.

Rosenzweig, M.L. 1975. *Species Diversity in Space and Time*. Cambridge University Press, Cambridge, UK.

Saino, N., Calza, S. & Møller, A.P. 1997. Immunocompetence of nestling barn swallows (*Hirundo rustica*) in relation to brood size and parental effort. *J. Anim. Ecol.* **6**: 827–836.

Sheldon, F.H. & Winkler, D.W. 1993. Intergeneric phylogenetic relationships of swallows estimated by DNA-DNA hybridization. *Auk* **110**: 798–824.

Sibley, C.G. & Ahlquist, J.E. 1990. *Phylogeny and Classification of Birds*. Yale University Press, New Haven, CT.

Soler, J.J., Møller, A.P., Soler, M. & Martínez, J.G. 1999. Interactions between a brood parasite and its host in relation to parasitism and immune defence. *Evol. Ecol. Res.* **1**: 189–210.

Sorci, G., Massot, M. & Clobert, J. 1994. Maternal parasite load increases sprint speed and philopatry in female offspring of the common lizard. *Am. Nat.* **144**: 153–164.

Thompson, J.N. 1994. *The Coevolutionary Process*. University of Chicago Press, Chicago, IL and London, UK.

Tripet, F., Christe, P. & Møller, A.P. 2002. The importance of host sociality for parasite specialization and speciation: a comparative study of avian hosts and their fleas (Siphonaptera: Ceratophyllidae). *J. Anim. Ecol.* **71**: 735–748.

Van Baalen, M. & Hochberg, M. 2001. Dispersal in antagonistic interactions. In: *Dispersal* (J. Clobert, J. D. Nichols, E. Danchin & A. Dhondt, eds), pp. 299–310. Oxford University Press, Oxford, UK.

Van Vuren, D. 1996. Ectoparasites, fitness and social behaviour of yellow-bellied marmots. *Ethology* **102**: 686–694.

Received 4 December 2002; revised 23 July 2003; accepted 15 October 2003

Appendix Information on nestling T-cell response (mm), sample size, number of breeding habitats defined as the number of habitats listed in Cramp & Simmons (1977–1994), migration distance from breeding area to wintering area (in multiple of 500 km), nest site (0, open; 1, hole), coloniality (0, solitary; 1, max. 10 pairs in colony; 2, max. 100 pairs in colony; 3, max. 1000 pairs in colony), and body mass (g) for bird species included in the present study.

Species	T-cell response (mm)	No. nestlings	No. breeding habitats	Migration	Nest site	Coloniality	Body mass (g)	Natal dispersal (km)	Breeding dispersal (km)
Acrocephalus scirpaceus	0.395	4	15	12	0	0	12.3	5.22	2.94
Alauda arvensis	0.379	9	10	3	0	0	42.7	0.30	0.17
Apus apus	1.902	4	5	12	1	2	37.6	11.47	2.63
Carduelis chloris	0.829	14	9	0	0	0	27.8	0.95	0.73
Clamator glandarius	1.193	27	7	4	0	0	167.0	–	–
Columba livia	2.808	8	4	0	1	2	393.0	–	–
Columba palumbus	2.261	6	8	1	0	0	490.0	2.28	1.81
Corvus corone	2.141	16	11	0	0	0	570	3.24	0.93
Corvus frugilegus	2.685	7	4	1	0	3	488.0	1.96	0.65
Corvus monedula	2.053	10	2	0	1	2	246.0	2.13	0.72
Cuculus canorus	1.130	1	15	12	0	0	106.0	–	–
Delichon urbica	2.186	61	4	12	0	3	14.5	3.19	0.69
Emberiza citrinella	0.628	4	12	1	0	0	26.5	2.03	0.16
Erithacus rubecula	1.238	3	3	1	1	0	18.2	0.57	0.36
Falco tinnunculus	2.348	16	14	2	0	0	186.0	14.01	1.69
Fringilla coelebs	0.727	7	8	2	0	0	21.9	0.79	0.30
Hirundo daurica	0.940	23	2	3	0	0	19.0	–	–
Hirundo rustica	1.265	1073	4	12	0	2	16.2	3.19	0.56
Lanius excubitor	1.297	3	7	2	0	0	62.1	–	–
Luscinia svecica	0.430	71	7	10	0	0	17.0	–	–
Motacilla alba	1.047	9	11	5	1	0	21.0	5.16	0.89
Motacilla cinerea	1.488	1	10	6	1	0	18.0	15.80	–
Muscicapa striata	1.398	9	13	12	0	0	14.6	3.38	0.80
Parus caeruleus	0.543	13	8	0	1	0	13.3	0.80	0.23
Parus major	0.625	25	8	0	1	0	19.0	0.80	0.25
Passer domesticus	1.328	12	10	0	1	1	28.0	0.21	0.15
Passer montanus	2.810	9	4	1	1	1	22.0	0.72	0.28
Phoenicurus ochruros	0.932	16	3	3	1	0	16.4	–	–
Phoenicurus phoenicurus	1.132	8	8	12	1	0	14.7	3.87	–
Phylloscopus bonelli	0.341	8	3	8	0	0	7.1	–	–
Pica pica	1.556	83	7	0	0	0	189.0	1.03	0.25
Pyrrhocorax graculus	1.659	9	2	0	0	1	245.0	–	–
Riparia riparia	0.990	78	7	13	1	3	14.6	6.65	1.22
Saxicola rubetra	0.206	6	10	12	0	0	16.9	–	–
Saxicola torquata	0.635	15	9	2	0	0	14.4	–	–
Serinus serinus	0.619	84	4	1	0	0	11.2	–	–
Sitta europaea	1.384	5	6	0	1	0	22.0	1.80	0.25
Streptopelia decaocto	2.455	5	5	0	0	0	152.0	2.50	0.57
Sturnus vulgaris	1.230	3	10	2	1	0	84.7	1.10	0.27
Sylvia atricapilla	0.933	6	12	5	0	0	15.5	17.54	8.03
Sylvia cantillans	0.597	6	10	9	0	0	8.5	–	–
Sylvia communis	0.512	8	13	12	0	0	14.5	2.82	1.15
Sylvia melanocephala	0.735	8	6	1	0	0	11.5	–	–
Sylvia undata	0.348	3	5	0	0	0	9.5	–	–
Troglodytes troglodytes	0.625	5	14	3	0	0	8.9	1.17	0.53
Turdus merula	1.353	27	11	0	0	0	113.0	0.26	0.22