Repeatability of cell-mediated and innate immunity, and other fitness-related traits, in the Grey Partridge

Marco Cucco, Giorgio Malacarne, Roberta Ottonelli, and Mauro Patrone

Abstract: Immunocompetence is considered a reliable indicator of general body condition and ultimately of fitness. It has been suggested that, as a parameter subjected to intense directional selection, the level of additive genetic variance expressed should be reduced; on the other hand, theoretical models of host–parasite coevolution assume that variation in parasite resistance has a genetic basis. Contradictory results have been reported in birds, since the heritability of immune responses varies from nil to high. In this study of Grey Partridge (*Perdix perdix* (L., 1758)), we examined the heritability of immune condition (PHA reaction, an index of T-cell-mediated immunocompetence) and of some parameters traditionally considered important for chick survival, such as egg mass and chick growth. Two statistical approaches were used: parent–offspring regression and full-sibling intra-clutch repeatability. The repeatability of other parameters that reflect egg quality (egg proteins, lipids, and carotenoids) and of substances that confer innate immunity (lysozyme and avidin) was also investigated. In agreement with previous studies, we found nonsignificant heritability for cell-mediated immunocompetence. In contrast, there were significant repeatabilities of chick mass and several egg characteristics (mass, size, total proteins), while lipid and carotenoid concentrations were not repeatable. For the first time in birds, we found significant repeatability of two molecules, lysozyme and avidin, that confer innate immunity to the developing embryo.

Résumé : L'immunocompétence est considérée comme un indicateur fiable de la condition corporelle globale et, en fin de compte, de la fitness. On a suggéré que, puisque c'est une variable sujette à une intense sélection directionnelle, le niveau de variance additive génétique exprimée devrait être réduit; par ailleurs, les modèles théoriques de coévolution hôte–parasite présupposent que la variation de la résistance du parasite a une base génétique. Les résultats signalés chez les oiseaux sont contradictoires, puisque l'héritabilité des réactions immunitaires y varie de nulle à forte. Dans notre étude sur la perdrix grise (*Perdix perdix* (L., 1758)), nous examinons l'héritabilité de la condition immunitaire (réaction PHA, un indice de l'immunocompétence reliée aux cellules T) et de quelques variables généralement considérées comme importantes pour la survie des poussins, telles que la masse des oeufs et la croissance des poussins. Nous avons utilisé deux méthodologies statistiques : la régression parents–rejetons et la répétabilité chez les vrais frères et soeurs dans une même couvée. Nous avons aussi examiné la répétabilité d'autres variables qui sont reliées à la qualité des oeufs (protéines, lipides et caroténoïdes des oeufs) et des substances qui confèrent une immunité innée (lysozyme et avidine). En accord avec les études antérieures, nous trouvons une héritabilité non significative de l'immunocompétence reliée aux cellules. En revanche, il y a une répétabilité significative de la masse des poussins et de plusieurs caractéristiques des oeufs (de la masse, de la taille et des protéines totales, mais pas des lipides et des caroténoïdes). Pour la première fois chez les oiseaux, nous trouvons une répétabilité significative chez deux molécules, le lysozyme et l'avidine, qui confèrent une immunité innée chez les embryons en développement.

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Introduction

Survival to adulthood is one of the most important determinants of lifetime reproductive success in animals (Clutton-Brock 1988). The conditions experienced during ontogeny in the different species are of critical importance, since they affect the developmental trajectories that lead to different survival perspectives of offspring (Merilä 1996).

In birds, conditions during the nesting stage have an important impact on subsequent fledging (Merilä 1996). Moreover, capture–recapture data show that chicks in better condition have a higher likelihood of survival and future reproduction (Perrins 1965). Within the developmental process, environmental factors (especially nutritional ones) are thought to be of primary importance for body condition, even if genetic components of offspring condition and viability parameters have been detected (Merilä 1996).

Maternal effects represent a peculiar environmental source of phenotypic variance in early-life traits (Mousseau and Fox 1998); indeed, they influence birth mass, birth date, and natal litter size in many mammalian species (Wilson et al.

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2005). Oviparous animals show a specific early-maternal effect in the form of substances that accumulate in the egg. Apart from the macronutrients (proteins and lipids) necessary for growth, micronutrients and molecules such as hormones, vitamins, and carotenoids play an important role in the physiological and behavioural maturation of individuals (Minvielle and Oguz 2002; Royle et al. 2003; Williams 2005). Proteins linked to innate immunity (immunoglobulin, lysozyme, etc.) are other substances transferred from mother to egg that influence the future prospects of chick survival (Saino et al. 2002).

Immunocompetence, like all phenotypic traits, shows genetic and environmental components of variance. Recent studies in birds have tried to partition the relative contribution of maternal, environmental, and genetic effects for several immune, humoral, and cellular elements (Lamont 1998; Hõrak et al. 2002; Råberg et al. 2003).

Maternal effects are of fundamental importance for defence against diseases. Mothers allocate several molecules to their eggs that protect the embryo from pathogen attacks (lysozyme and avidin, among others) or that are critical for chick survival in the first days of life (immunoglobulin) when the largely immune-deficient young are highly susceptible to infectious diseases which can affect both survival and the expression of many life-history traits (Saino et al. 2002). Chicks progressively acquire their autonomous immunity (Pastoret et al. 1998) via specific humoral and cellular immunities, and early environmental (mainly nutritional) conditions can play a role in the acquisition of good immunocompetence. Indeed, the chick's early diet can influence both cell-mediated responses and humoral responses to antigens, as demonstrated with fasting experiments (Alonso-Alvarez and Tella 2001; but see Råberg et al. 2003 for different results) and differential nutrition studies (Tengerdy et al. 1990; Fenoglio et al. 2002; Koutsos et al. 2003; Ottonelli 2005).

Finally, recent studies have attempted to identify the genetic component of this pool of phenotypes. However, perhaps because of the heterogeneity of approaches and the different species studied, the results are varied and apparently contradictory, ranging from nearly nil heritability to very substantial heritability of immune reactivity. In Red Jungle Fowl (*Gallus gallus* (L., 1758)), resistance to disease and certain parasites were found to be highly heritable (Hutt 1949; Hartman 1985). Great Tit (*Parus major* L., 1758) nestlings exhibited variation in heritability of cell-mediated immunity (Brinkhof et al. 1999), while Barn Owl (*Tyto alba* (Scopoli, 1769)) siblings showed similar humoral immunity (Roulin et al. 2000). In Blue Tit (*Parus caeruleus* (L., 1758)), the heritability of responsiveness was low for the reaction to diphtheria but significant for tetanus (Råberg et al. 2003). Interestingly, no significant additive genetic variance in immune response was found in Common House-Martin (*Delichon urbica* (L., 1758); Christe et al. 2000), Pied Flycatcher (*Ficedula hypoleuca* (Pallas, 1764); Soler et al. 2003), and American Kestrel (*Falco sparverius* L., 1758; Tella et al. 2000), suggesting low or nil heritability. In these cases where the population variability in immunity is due to the environmental component, intense directional selection has been invoked as the cause of the reduced level of additive genetic variance (Christe et al. 2000; Tella et al. 2000). On the whole, these studies indicate that humoral antibody responsiveness has higher heritability than cell-mediated responsiveness, but the reasons for this difference are unknown (Råberg et al. 2003).

Briefly, studies of both the responsiveness to nonpathogenic antigens and the resistance to particular parasites show that there is often, but not always, a considerable amount of genetic variation in immune function. Genetic variation in immune function is the foundation of theoretical models of host–parasite coevolution that assume a partially genetic basis of the variability in susceptibility among hosts (Brinkhof et al. 1999). Since this trait is subjected to intense directional selection, its low heritability could be related to its positive contribution to fitness; i.e., strong and constant directional selection makes the depletion of genetic variation likely, with all remaining variation being environmentally determined (Roff 1997, but see Merilä and Sheldon 2000). On the other hand, since the immune system defends the organism against various parasites, a certain level of heritability is predicted by coevolutionary hypotheses (Hamilton and Zuk 1982; Ligon 1999).

Besides immunocompetence, other factors important for chick condition and survival, such as egg mass and body size, have been studied with regard to gene–environment partitioning and evolvability of phenotypic traits (Merilä et al. 2001). Contrary to the conflicting results concerning immune response, there is agreement that egg mass is a highly repeatable trait (Christians 2002). Egg mass has been frequently related to chick mass and survival (Lack 1968); it varies considerably in birds, but only a small amount of variation across species is attributable to environmental conditions. Studies on the heritability of body size at hatching also show homogeneous values, ranging from intermediate to high (Cabezas-Diaz et al. 2005), that are dependent on ecological variables (Christe et al. 2000).

In our research on the repeatability of immune response and other fitness-related traits, we used Grey Partridge (*Perdix perdix* (L., 1758)) as the study model. Two aspects make it an interesting model: (1) in precocious species, unlike altricial ones, the maternal effects are limited to investment in eggs so that conditions derived from parental feeding and care are not confounding variables; (2) in this study, the breeding pairs were separated so that extra-pair paternity could be excluded and the reliability of full-sibling analyses guaranteed (Merilä and Sheldon 2001).

Here we examine the repeatability of immune condition and several parameters traditionally considered important for chick survival, such as egg mass and chick growth. Two statistical approaches were used: (1) parent–offspring regression and (2) intra-clutch repeatability. Results on the repeatability of other parameters reflecting egg quality (egg proteins, lipids, and carotenoids; Minvielle and Oguz 2002; Royle et al. 2003; Williams 2005) are also reported, and repeatability estimates of substances that confer innate immunity (lysozyme and avidin) are reported for the first time in birds.

Methods

The field study was conducted on captive Grey Partridges reared at a game farm in San Giuliano Nuovo, Alessandria (northwestern Italy), during the 2002–2003 breeding seasons.

The Grey Partridges were part of a stock reared in captivity for at least 10 years. Phylogenetic analyses of mitochondrial DNA show that they pertain to the *Perdix perdix perdix* (L., 1758) major clade of Western Europe, excluding the *Perdix perdix lucida* (Altum, 1894) major clade of Eastern Europe (A. Negri and E. Randi, personal communication). We studied 32 breeding pairs in 2002 and another 32 pairs in 2003. All breeding birds were 1 year old. During the study, each female was paired with a male, and both birds were placed in outdoor breeding pens (4 m long × 1 m wide × 0.5 m high) and maintained under natural light and temperature conditions. From April to June, the 64 hens laid a total of 1040 eggs in 2002 and 1037 eggs in 2003.

For each individual, we measured body mass with an electronic balance (±0.01 g accuracy) and T-cell-mediated immune response with the PHA test (Smits et al. 1999) before the beginning of the laying period. PHA has a mitogenic effect on T lymphocytes, and the injection stimulates macrophage infiltration and dense perivascular accumulation of lymphocytes; the response to PHA inoculation has been shown to be a reliable indicator of one component of immunocompetence (Lochmiller et al. 1993). Birds were injected intradermally in the wing web (the patagium) with 0.25 mg of PHA (Sigma L-8754) diluted in 0.05 mL phosphate-buffered saline solution (PBS). The thickness of the wing webs was measured before and 24 h after injection in inoculated sites using a spessimeter (Alpha spa, Milan, Italy) with an accuracy of ±0.01 mm. Swelling of the wing web (T-cell-mediated immune response) was calculated as the difference in thickness of the wing web prior to and 24 h after injection.

Egg collection and measurements

When females started laying, all pens were inspected daily to collect eggs (in the afternoon, all within 1–5 h after laying). Using a nontoxic marker pen, we marked each egg with the female's code, the position in the laying order, and the date of laying. Just after collection, we weighed the eggs with an electronic balance (±0.01 g accuracy). Egg length and breadth were measured with a calliper (±0.1 mm accuracy).

One thousand and seventy-seven of the 2077 eggs were incubated for 26 days in a commercial incubator at 37.5 °C and 60% humidity, while 200 eggs (100 from each year) from 40 different females (5 eggs each, specifically the 5th, 7th, 10th, 13th, and 16th in the laying sequence) were brought to the laboratory for chemical analyses. In the laboratory, the eggs were carefully separated into their constituent parts (shell, albumen, and yolk; all weighed to the nearest ±0.01 g). The yolk was then homogenized and stored at –20 °C until analysis, while the albumen was frozen without centrifugation. The yolk was chemically analysed to assess lipids, proteins, β-carotene, and total carotenoid concentration, while the albumen was used to assess lysozyme and avidin contents.

The lipids were extracted with a Soxhlet apparatus, and protein content was assessed with the Kjeldahl method.

In fresh eggs, the lean mass was evaluated non-invasively by measurement of electroconductivity with the TOBEC apparatus (EM-SCAN Inc., Springfield, Illinois, USA). This method estimates lean mass, since the contribution of lipid tissue to conductivity is negligible (Walsberg 1988; Castro et al. 1990). As electrical conductivity is sensitive to temperature, the eggs were measured at a constant temperature of about 37 °C after warming in an incubator. The lean mass index was significantly related (Pearson's correlation, $r = 0.872$, $P < 0.001$, $n = 99$) to true lean mass, as measured in the sample of 99 eggs chemically analyzed with the Soxhlet apparatus.

β-Carotene concentrations in egg yolk were analysed by high-performance liquid chromatography (HPLC). An aliquot of yolk (0.2–0.5 g) was homogenized in 2 mL of a 1:1 (*v*/*v*) mixture of 5% NaCl solution – ethanol, followed by the addition of 3 mL of hexane and further homogenization for 3 min. After centrifugation, hexane was collected and the extraction was repeated twice. Hexane extracts were combined and evaporated under N_2, the residue was dissolved in 1 mL of a mixiture of methanol–dichloromethane (1:1, *v*/*v*) and centrifuged, and the supernatant was used for carotenoid determination. β-Carotene was determined by HPLC with a Waters TM Alliance 2695 Modul System (Millipore Corp., Bedford, Massachusetts, USA), using a Spherisorb type S3ODS2, 5 m C18, reverse-phase column, 25 mm (Phase Separation, Clwyd, UK), with a mobile phase of acetonitrile–methanol (85:15, by volume) and acetonitrile–dichloromethane–methanol (70:20:10, by volume) in gradient elution, and detection by absorbance at 445 nm (Waters 2996 photodiode array detector; Waters Chromatography, Milford, Massachusetts, USA). Peaks were identified by comparison with the retention times of carotenoid standards and integrated with EMPOWER software.

The concentration of total egg yolk carotenoids was measured spectrophotometrically. Egg yolk samples were added to a mixture of hexane–acetone–toluene–ethanol (10:7:7:6, by volume), and centrifuged at 10000 r/m for 10 min (10000 *g*). In the resulting supernatant, we determined the absorbance of the carotenoid peak at 450 nm using a Beckman Du-640 spectrophotometer. Carotenoid concentrations (μg/mL) were calibrated according to standard curves of β-carotene (Sigma).

Lysozyme activity was measured by the method of Osserman and Lawlor (1966) as follows: an agar gel with a dried strain of *Micrococcus lysodeikticus* (M-3770; Sigma), which is particularly sensitive to lysozyme activity, was inoculated with 25 μL of albumen. Standard dilutions of crystalline hen egg-white lysozyme (L-6876, Sigma) (25, 100, 500, and 1000 μg/mL) were run with each group of test samples. The plates were incubated at room temperature (24–26 °C) for 18 h, during which bacterial growth was inhibited in the area of the gel surrounding the albumen inoculation site. The diameters of the cleared zones are proportional to the logarithm of the lysozyme concentration. This area was measured using an ad hoc ruler and converted on a semi-logarithmic plot into hen egg lysozyme equivalents (HEL equivalents, expressed in μg/mL) according to the standard curve.

The biotin-binding capacity (active avidin) of samples from egg whites was measured using biotinylated insulin and biotinylated alkaline phosphatase (AP, Sigma). The 96-well plate was coated with biotinylated insulin (10 μg/mL) in sodium carbonate buffer (50 mmol/L, pH 9.6) at 37 °C for 2 h, followed by washing three times with PBS–Tween and blocking with 1% BSA in PBS (PBS–BSA). Egg-white samples were diluted 1:6 with 0.5% BSA in PBS, vortexed briefly, and incubated for 2 h at 25 °C. Duplicate samples were allowed to bind to biotinylated insulin at 37 °C for 1 h at

100 μL volume, followed by washing five times with PBS–Tween. Biotin saturated samples (biotin 17 mg/L, Sigma) and BSA were used as negative controls, and the assay was standardized with chicken avidin (Sigma) diluted to known concentrations. Biotinylated AP was used to probe the bound biotin-binding proteins diluted 1:3000 in PBS–BSA (1 h, 37 °C). Para-nitrophenyl phosphate (1 mg/mL, Sigma) was used as a signal molecule and absorbencies were measured at 405 nm with a Bio-Rad 450 Microplate reader.

Chick measurements

Hatching of incubated eggs was synchronous. To determine which chick hatched from which egg in the clutch, we incubated each egg in a small wire cage so that the hatched young were singly confined. Upon hatching, each chick was marked with a numbered plastic ring on its leg to allow subsequent identification.

We measured the body mass of chicks (±0.01 g accuracy) on the day of hatching and after 10, 21, and 60 days. We used the PHA test (as described for adults) to measure cell-mediated immunity on two occasions, on days 10 and 21.

Each chick had food and water available ad libitum. All young were raised together for 6 weeks in two heated pens in a nursery room maintained at 20.5 ± 2.0 °C under full-spectrum artificial light. After 6 weeks, the chicks were removed from the heated pens and placed in a large outdoor aviary.

Statistical analysis

Heritability and repeatability were estimated based on morphological and physiological characters.

We estimated narrow-sense heritabilities (h^2) using parent–offspring regressions, where heritability is the proportion of total variance attributable to the additive effects of genes. Heritability was estimated as twice the regression coefficient from this analysis (Falconer and MacKay 1996). The data used span 2 years and were part of a twin study examining the physiological effects of two different quantities of β-carotene in the diet (Ottonelli 2005). Thus, we calculated the residuals from an ANOVA with year and diet as factors and mass as the dependent variable. In a similar manner, we calculated the residuals with PHA immune responses as the dependent variables. These residuals were standardized to a mean of zero and variance of unity, and were used in all the statistical analyses.

An alternative heritability estimate (repeatability, r) was derived from a one-way ANOVA with pair as a factor and calculated as the intraclass correlation coefficient, assuming that all nestlings are full siblings (sibling analysis; Falconer and Mackay 1996). Both heritability estimates include common environmental effects and may be inflated when those effects explain significant variation, in particular sibling analyses (Falconer and Mackay 1996; Dingemanse et al. 2002). We estimated the repeatability (intraclass correlation coefficient) of egg characteristics (mass, length, breadth, proteins, lipids, lean mass, β-carotene, total carotenoids, lysozyme, avidin), chick mass (10 and 21 days after hatching), and cell-mediated immune response (10 and 21 days) according to the suggestions of Lessells and Boag (1987).

All analyses were performed with SYSTAT® version 11 (Systat Software Inc. 2004).

Table 1. Heritability (h^2) estimates based on morphological and physiological characters of Grey Partridge (*Perdix perdix*) parent–offspring interactions.

Variable	F	P	h^2	SE
Mother–offspring				
Mass at day				
10	6.880	0.010**	0.466	0.178
21	2.687	0.104	0.310	0.190
60	2.710	0.010**	0.381	0.141
Immune response at day				
10	0.163	0.69	–0.08	0.186
21	0.054	0.82	0.054	0.220
Father–offspring				
Mass at day				
10	0.234	0.630	0.096	0.200
21	0.018	0.893	–0.03	0.204
60	2.554	0.117	0.210	0.132
Immune response at day				
10	0.937	0.335	–0.19	0.194
21	1.699	0.196	0.266	0.204

**, $P \leq 0.01$.

Results

Heritability (parent–offspring regression, h^2)

Parent–offspring regressions yielded significant heritability estimates for chick mass in relation to maternal mass, while paternal masses were not related to those of the offspring (Table 1). The value of h^2 was rather high in the 10 day old chicks and decreased in older chicks.

The chick's immune reaction to PHA was not significantly related to that of the mother or to that of the father. The values of h^2 were very low or negative (Table 1).

Repeatability (intraclass correlation coefficient, r)

The repeatability values for egg and chick characteristics are reported in Table 2. The repeatability of egg mass and size was remarkably high, from 0.65 to 0.22 (Table 2). Among the main components of eggs, there was a high repeatability of lean mass and protein content, while lipid content did not reach a significant r value. Among the other analysed chemical components, the concentrations of β-carotene and total carotenoids did not differ among clutches, while there was a significant r value of lysozyme and avidin concentrations (Table 2).

The results of repeatability for chick mass and chick immune reaction to PHA were similar to those observed for parent–offspring regression, i.e., there was significant repeatability of chick mass, and the r value was higher in 10 day old chicks than in older chicks. In contrast, the immune reaction to PHA was not repeatable in Grey Partridge siblings (Table 2), even if there was within-individual repeatability of the PHA reaction at 10 and 21 days of age ($r = 0.496$, $P < 0.001$, $n = 295$).

Allometric relationships

Since there could be a possible correlation between body mass and immune reaction (Tella et al. 2000), we tested for an allometric increase of PHA reaction with body mass.

Table 2. Repeatability (intraclass correlation coefficient, *r*) of reproductive traits in the Grey Partridge.

Variable	*F*	df	*P*	*r*
Egg mass	60.8	62, 2009	0.001***	0.65
Egg length	34.3	62, 2011	0.001***	0.51
Egg breadth	9.92	62, 2010	0.001***	0.22
Protein content	1.64	34, 83	0.037*	0.16
Lipid content	1.48	33, 83	0.077	0.13
Lean mass	10.4	25, 489	0.001***	0.34
β-Carotene concentration	0.94	39, 157	0.584	–0.014
Total carotenoid concentration	1.54	18, 80	0.097	0.10
Lysozyme concentration	1.86	18, 80	0.032*	0.15
Avidin concentration	2.34	20, 62	0.006**	0.25
Chick mass at day				
10	3.24	56, 390	0.001***	0.23
21	1.88	54, 245	0.001***	0.14
60	1.64	42, 98	0.024*	0.168
Immune response at day				
10	1.18	56, 343	0.192	0.026
21	1.06	52, 236	0.380	0.011

*, $P < 0.05$; **, $P < 0.01$; ***, $P \leq 0.001$.

However, body mass and immune reaction were not significantly related in either 10 day old chicks (Pearson's correlation, $r = 0.074$, $P = 0.14$ (ns), $n = 401$; Fig. 1*a*) or 21 day old chicks ($r = 0.082$, $P = 0.16$ (ns), $n = 290$; Fig. 1*b*).

We also tested whether lysozyme (Fig. 2) and avidin (Fig. 3) concentrations varied in relation to egg mass. Lysozyme concentration was not significantly related to egg mass (Pearson's correlation, $r = 0.042$, $P = 0.68$ (ns), $n = 100$), and neither was avidin concentration ($r = 0.017$, $P = 0.88$ (ns), $n = 89$).

Egg mass significantly affected the mass that was later attained by the chicks (Pearson's correlation between mean egg mass and mean chick mass of each female; at age 10 days: $r = 0.551$, $P < 0.001$, $n = 64$; at age 21 days: $r = 0.483$, $P < 0.001$, $n = 64$; only marginally at age 60 days: $r = 0.263$, $P = 0.08$ (ns), $n = 64$).

Discussion

This study of the Grey Partridge shows high repeatability of morphological parameters traditionally considered important for chick survival, such as egg mass and chick growth, as well as of other parameters reflecting egg quality (egg proteins and size). However, cell-mediated immunocompetence was not very repeatable. Substances that confer innate immunity (lysozyme and avidin), examined for the first time in birds, were found to be highly repeatable.

Morphological traits

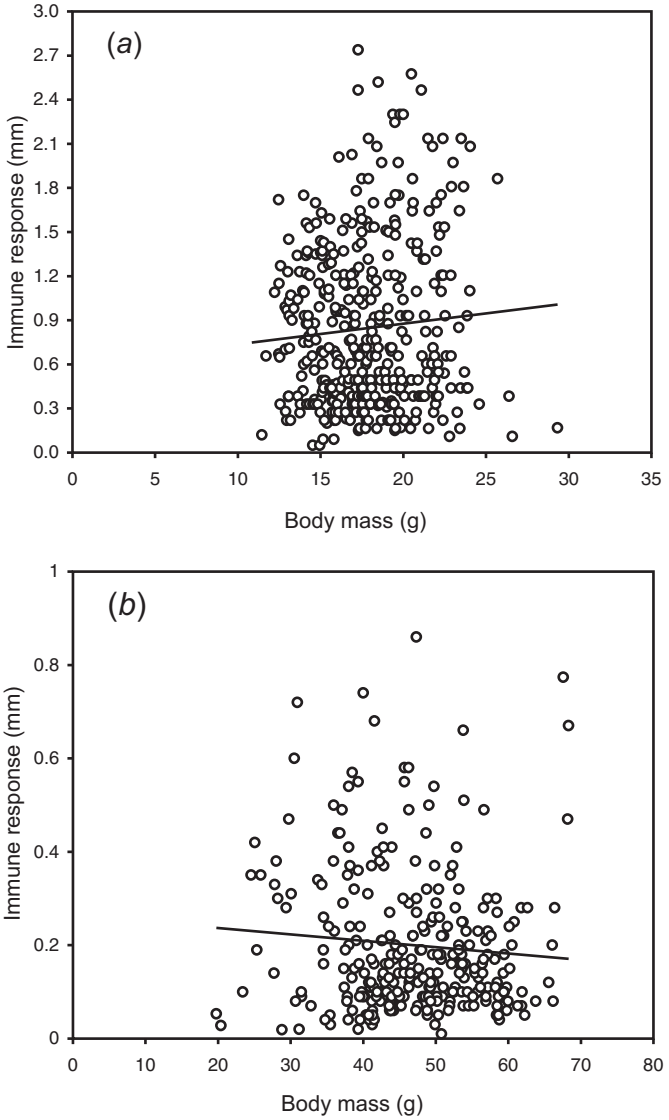
Morphological traits were highly heritable (chick mass) or repeatable (egg mass, egg size, and chick mass). The values were comparable with estimates from other bird species (Christians 2002; Merilä and Sheldon 2001), particularly the phylogenetically related Red Grouse (*Lagopus lagopus* (L., 1758)) and Red-legged Partridge (*Alectoris rufa* (L., 1758)) (Moss and Watson 1982; Cabezas-Diaz et al. 2005). Indeed, low heritabilities of morphological traits have rarely been reported (Christe et al. 2000). As expected, egg mass had a significant effect on the mass attained by chicks in the first few days after hatching and later.

The results of mother–offspring regression differed from those of father–offspring regression, the characters of chicks being significantly related to those of the mother but not to those of the father. A sex difference in the amount of heritable genetic variation has been shown in several species, with generally higher values in females (Jensen et al. 2003). This suggests that higher heritability in females than in males may be common in birds, and that sex-specific additive genetic variances and covariances (ignored in most studies) should be included when making predictions of evolutionary changes from standard quantitative genetic models (Jensen et al. 2003).

In addition to the high repeatability of egg size and mass, we found a significant repeatability of the major egg components, the total egg proteins and lean mass (with the exception of lipid content, which was not significant). This result is in line with previous studies on repeatability of egg composition (Carey 1996; Christians and Williams 2001*a*, 2001*b*). The low repeatability of egg lipid content suggests that any limitations on egg production are manifested in reductions of overall egg mass and proportional protein contents, and only to a lesser extent in variation of the lipid composition of the eggs (Hipfner et al. 2002).

The repeatability of β-carotene and carotenoid concentrations in the eggs was low and not statistically significant. This indicates that different females accumulated carotenoids in similar concentrations, as recently shown in the Moorhen (Fenoglio et al. 2001). Several physiological processes depend on or are regulated by carotenoids (Surai 2002). Carotenoids act as scavengers of reactive oxygen species, protecting biological molecules from oxidative damage, and they play an important role in the regulation of immune function (Chew 1993; Edge et al. 1997; Olson and Owens 1998; Blount et al. 2000; Møller et al. 2000; Surai et al. 2001). Carotenoids may play a role in protection against oxidative stress during the embryonic and early post-hatching stages, and they may

Fig. 1. Relationship between mass and immune response of Grey Partridge (*Perdix perdix*) chicks to PHA injection. (*a*) 10 day old chicks; (*b*) 21 day old chicks.



promote maturation and functioning of the embryonic immune system, which is probably crucial to offspring survival because immune defence tends to be weak soon after birth (Tizard 1991; Pastoret et al. 1998).

Immunity

In this study, we evaluated the heritability of T-cell-mediated immune response using both parent–offspring regression and full-sibling analyses. The results indicate a nonsignificant genetic additive component for this trait. There are only a few previous studies of heritability of immune responsiveness in birds. Roulin et al. (2000) found statistically significant heritability of antibody responsiveness to SRBC in a population of Barn Owls, and Brinkhof et al. (1999) found heritability of cell-mediated responsiveness to PHA in the Great Tit. In contrast, Christe et al. (2000), Tella et al. (2000), and Soler et al. (2003) found that the heritability of this trait in populations of the Common House-Martin, American Kestrel, and Pied Flycatcher, respectively, was very low and not statistically significant. A few studies have also investigated the heritability of responses to particular parasites and the estimates were generally rather high (Møller 1990; Boulinier et al. 1997; Smith et al. 1999); a difference among parasites was demonstrated by Råberg et al. (2003) who found that only the responsiveness to tetanus (and not to diphtheria) had a strong genetic component. On the whole, the data indicate that antibody responsiveness has higher heritability than cell-mediated responsiveness (Råberg et al. 2003).

In this study, we found significant repeatability of substances that confer innate immunity (lysozyme and avidin). Lysozyme is an enzyme that attacks the bacterial cell wall,

Fig. 2. Relationship between egg mass and concentration of lysozyme (measured as hen egg lysozyme equivalents (HEL equivalents)) in Grey Partridge chicks. Lysozyme concentrations were log transformed.

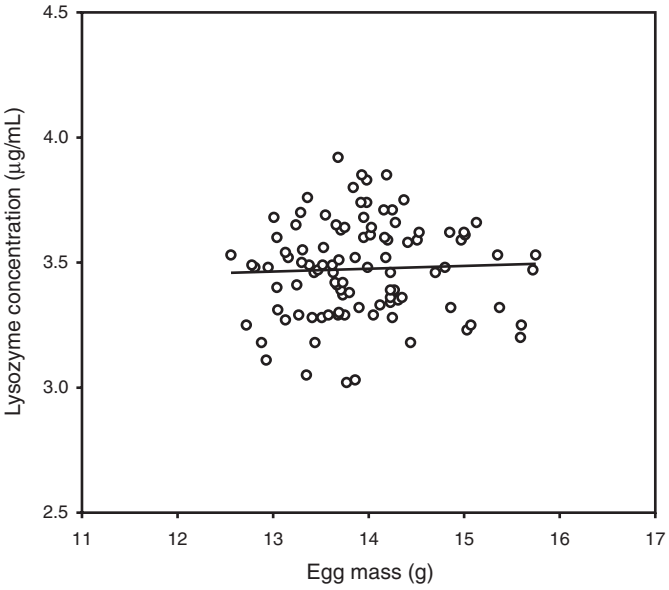
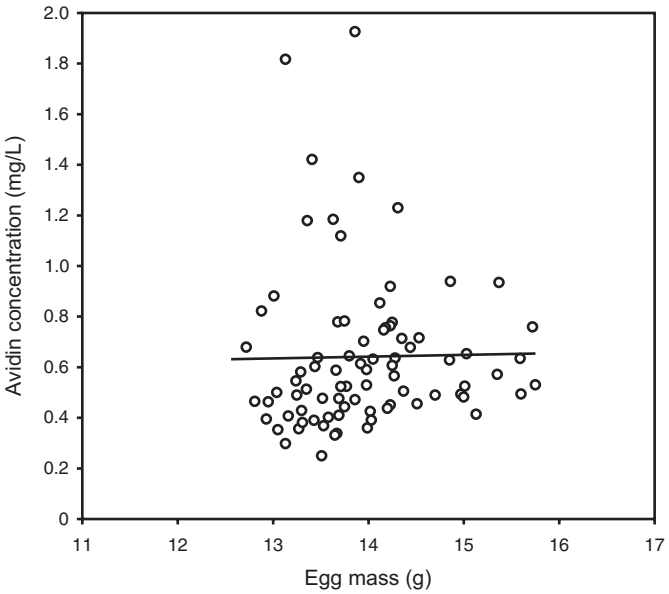


Fig. 3. Relationship between egg mass and concentration of avidin in Grey Partridge chicks.



degrading it by cleaving the sugar backbone of the peptidoglycan component. Specifically, lysozyme adds water to (hydrolyzes) the glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine. Avidin is a strongly basic glycoprotein synthesized in the female oviduct and deposited in the albumen fraction of eggs. It tightly and specifically binds the water-soluble vitamin H (biotin), an essential factor for micro-organisms.

There have been only a few studies on the heritability of lysozyme (e.g., Lund et al. 1995; Balfry et al. 1997; Fevolden et al. 1999; Johnson et al. 2003 on adult trout; Kurtz and Sauer 1999 on the scorpion fly; Pal et al. 1996 on the guineafowl) and, to the best of our knowledge, virtually none on avidin. Our study provides new data suggesting that the ability to accumulate lysozyme and avidin in eggs may be characters with a genetic component, indicating that innate immunity may have both an environmental component (Ottonelli 2005) and a hereditary component.

Further research is needed to clarify both the physiological mechanisms that might lead to variance in egg quality and, in a long-term perspective, the adaptive value of differences among individuals.

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