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# Do experiments with captive non-domesticated animals make sense without population field studies? A case study with blue tits' breeding time

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A complete understanding of the spatio-temporal variation in phenotypic traits in natural populations requires a combination of long-term field studies with experiments using captive animals. Field studies allow the formulation of realistic hypotheses, but have the disadvantage that they do not allow the complete control of many potential confounding variables. Studies with captive animals allow tests of hypotheses that cannot be examined in the field, but have the disadvantage that artificial environments may provoke abnormal behaviour. Long-term studies that follow simultaneously captive and wild bird populations are rare. In a study lasting several years, we show here the unexpected patterns that two populations with a similar breeding time in the wild have non-overlapping breeding times in outdoor aviaries, and that two wild populations separated by a short geographical distance show differences in the expression of natural behaviour in captivity. The experimental design used is exceptional in the sense that the captive populations were held at similar latitudes and altitudes as the wild populations. Our case study shows that studies with captive animals can lead to wrong conclusions if they are carried out without population field studies, and without knowledge of the natural habits and habitats of the species involved. To examine the reliability of experiments with captive animals, comparisons with findings from population field studies are essential.

**Keywords:** blue tit; *Parus caeruleus*; seasonal breeding; captive animals; population studies; avian reproduction

## 1. INTRODUCTION

Combining long-term field studies with experiments using captive animals is crucial for a full understanding of the different sources of variation in phenotypic traits that occur in the natural populations. Field studies help us to create realistic hypotheses concerning phenomena that occur in natural populations. However, field studies do not allow a full control of the many factors that influence phenotypic traits in their natural environment. Studies with captive animals are important because they allow tests that cannot be carried out with field experiments (Visser & Lambrechts 1999). Experiments with captive animals have the disadvantage, however, that artificial environments that control for many environmental factors may provoke abnormal behaviour (Dawkins 1998; King 1999; Winkler & Leisler 1999). In addition, within a few generations, captive animals may become quite different from their wild counterparts if the captive animals are exposed to new selection regimes (Kohane & Parsons 1988).

In non-domesticated birds, reproductive and physiological traits can differ considerably between captive and wild individuals (Kendeigh 1941; Kern 1972; Murton & Westwood 1977; Wingfield 1980; Silverin & Westin 1995). Furthermore, captive non-domesticated birds rarely breed

(Wingfield 1980; Silverin & Westin 1995; but see Meijer & Schwabl 1989; Gwinner 1996). In blue tits (*Parus caeruleus*) from the Mediterranean region, captive breeding individuals have been studied in the framework of long-term field studies (Blondel *et al.* 1990, 1993; Lambrechts & Dias 1993; Dias & Blondel 1996a,b; Lambrechts *et al.* 1997a). In the wild, leaf-eating caterpillars are a key prey for nestling tits (Perrins 1991; Pascual & Peris 1992; Blondel *et al.* 1991, 1998) and the peak-date of caterpillar availability is considered to be an important source of selection of the timing of reproduction (Perrins 1991; Van Noordwijk *et al.* 1995; Dias & Blondel 1996a). Blue tits from mainland southern France and Corsica occupy two distinct habitat types, i.e. broad-leaved deciduous oak woodland (*Quercus pubescens*) with an early peak-date of caterpillar abundance in May versus evergreen oak woodland (*Q. ilex*) with a late peak-date of caterpillars in June (Zandt *et al.* 1990; Blondel *et al.* 1993; Dias & Blondel 1996a; Lambrechts *et al.* 1997a). On the mainland, blue tit breeding time is nicely adjusted to the peak-date of caterpillars in broad-leaved deciduous woodland and they breed about ten days later in evergreen oak woodland. Gene flow from broad-leaved deciduous woodland towards evergreen woodland prevents mainland blue tits from adjusting their timing of reproduction to the late optimal breeding time in evergreen woods (Dias *et al.*

1311

1996). On Corsica, however, population differences in breeding time are much larger. Some blue tit populations breed at least one month earlier than others. In one valley (Corsica-Pirio) dominated by evergreen oak woodland the nestling stage occurs in June and matches the late peak-date of caterpillars in evergreen woods. In a nearby valley (Corsica-Muro) where broad-leaved deciduous oak trees are common, the nestling stage occurs in May and matches the early peak-date of caterpillars in broad-leaved deciduous oak habitat (Lambrechts *et al.* 1997*a*). An additional ten-year study with captive blue tits showed that large population differences in breeding time between the mainland and Corsica-Pirio blue tits are preserved in large outdoor aviaries on the European mainland (Blondel *et al.* 1990; Lambrechts & Dias 1993; Lambrechts *et al.* 1996, 1997*b*).

Here we present the results of a five-year study of breeding time with captive Corsica-Muro blue tits held in the same aviaries as in former studies with captive blue tits (see above). The breeding times obtained for wild and captive Muro blue tits are compared with those obtained with the mainland and Corsica-Pirio blue tits. We show that two populations with a similar timing of reproduction in the field, and similar responses to inter-year fluctuations in the date of maximum caterpillar availability, have non-overlapping breeding times in captivity where we controlled for several potential confounding variables. We also show that two populations that are separated by a short geographical distance in the wild (25 km) show considerable differences in the development of natural behaviour in captivity. Our findings have important implications for the interpretation of results from laboratory experiments with non-domesticated animals carried out without knowledge of what is going on in the natural populations.

## 2. MATERIAL AND METHODS

### (a) *Field*

The three study populations considered (mainland southern France, oak *Q. pubescens*, 43°40′ N, 03°35′ E versus Corsica-Muro, oak *Q. pubescens*, 42°36′ N, 08°58′ E; Corsica-Pirio, oak *Q. ilex*, 42°24′ N, 08°44′ E, see above; Dias & Blondel 1996*a*; Lambrechts *et al.* 1997*a*) were simultaneously followed from 1993 to 1998 following basic protocols that have been applied in blue tit study populations in the Mediterranean region for more than 20 years (Blondel & Isenmann 1979; Blondel *et al.* 1993; Dias & Blondel 1996*a*,*b*). Because the different stages of reproduction after egg laying are rather fixed, and blue tits rarely produce second clutches, the average onset of egg laying of first clutches provides reliable information about the timing of reproduction at the population level. Lay dates starting 30 days after the start of the first clutch of the year (9.5%) were considered as replacement or second clutches, and therefore not considered in the analyses.

Caterpillar frass fall collected under trees was estimated simultaneously in both populations from 1993 onwards following protocols described by Zandt *et al.* (1990) and Blondel *et al.* (1991, 1998). These measures provide good estimates of the peak-date of caterpillar availability (Zandt 1994). Because of bad weather, the peak-date of caterpillar frass fall could not be determined for the mainland study plot in 1998.

### (b) *Aviaries*

Captive non-domesticated blue tits from the Mediterranean region can breed (nest construction, egg laying, feeding offspring) with success in large outdoor aviaries provided with a nest-box and with one or two evergreen trees (*Q. ilex*) and food *ad libitum* (see Blondel *et al.* 1990; Lambrechts & Dias 1993; Lambrechts *et al.* 1996, 1997*b*). The breeding conditions were standardized in the sense that pairs of different populations were exposed to the same tree species, the same altitude and latitude, the same amount of space and the same food. Captive mainland and Pirio blue tits were studied from 1986. The Muro blue tits were followed in captivity from 1994. The aviaries, containing one pair per aviary, were situated on the mainland at similar altitudes and latitudes (43°38′ N, 03°52′ E) as the wild populations (see above), and were therefore exposed to similar microclimate and vernal changes in day length (Lambrechts *et al.* 1996, 1997*b*). All birds were in captivity at least four months before the start of egg laying, and some reproduced in different years. Twelve captive Muro pairs provided 19 nest construction dates and ten of them gave 14 laying dates. Twelve captive Pirio pairs provided 16 nest construction dates and 17 egg-laying dates. Ten captive mainland pairs provided 13 nest construction dates and 13 laying dates. Data from different years were lumped because individual captive females show little between-year fluctuations in onset of egg laying (Blondel *et al.* 1990; Lambrechts & Dias 1993). Statistical analyses were carried out using SAS, procedure GLM (SAS 1989). Averages with standard deviations, sample sizes ($n$) and probabilities of statistical tests ($p$) are provided.

## 3. RESULTS

### (a) *Field*

During the six-year study period, the average onset of egg laying in the field differed highly significantly between the three study sites. Blue tits from Corsica-Pirio bred more than one month later than the blue tits from the other two study sites (mainland: 6 April ± 9.3 days, $n = 343$, versus Muro: 7 April ± 10.0 days, $n = 163$, versus Pirio: 15 May ± 6.2 days, $n = 375$; figures 1 and 2). Effects of year ($F_{5,863} = 86.31$, $p < 0.0001$), study site ($F_{2,863} = 3577.26$, $p < 0.0001$), and the interaction site × year ($F_{10,863} = 17.76$, $p < 0.0001$) on the onset of egg laying were highly significant. Excluding the Pirio data from the analyses resulted in a significant year effect ($F_{5,494} = 83.05$, $p < 0.0001$), a significant interaction year × site effect ($F_{5,494} = 3.25$, $p < 0.01$), and a non-significant study-site effect ($F_{1,494} = 3.65$, $p < 0.057$).

In the three study populations, year effects could be attributed to inter-year fluctuations in the timing of maximum caterpillar availability. Across years, effects of the peak-date of caterpillar frass fall abundance ($F_{1,11} = 44.75$, $p < 0.0001$) and the study site (Pirio versus Muro versus mainland: $F_{2,11} = 4.92$, $p < 0.03$) on the average onset of egg laying were statistically significant. The interaction site × frass was statistically not significant ($F_{2,11} = 3.82$, $p = 0.055$). For the two study sites dominated by broad-leaved deciduous oak woodland (mainland versus Corsica-Muro), the effect of the peak-date of caterpillar abundance ($F_{1,7} = 37.79$, $p < 0.0001$) on the onset of first clutches was highly significant, but the effects of study site ($F_{1,7} = 0.00$, $p = 0.98$) and the interaction site × frass ($F_{1,7} = 0.03$, $p = 0.87$) on the onset of first clutches were not. The close link between onset of first
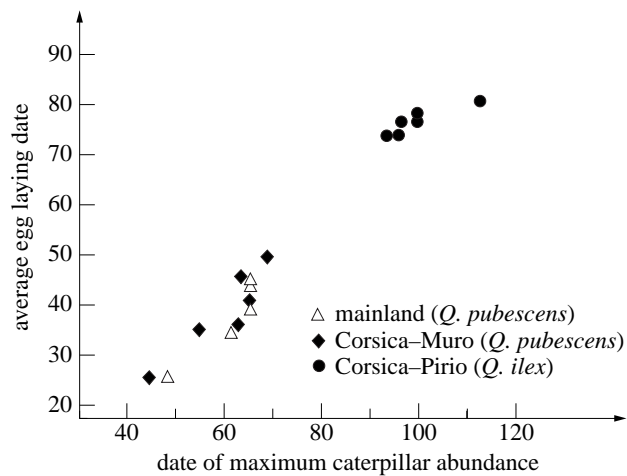
Figure 1. Onset of egg laying of first clutches in blue tits and the peak-date of caterpillar frass fall abundance across years (1993–1998) in three study plots (see §2), two plots dominated by broad-leaved deciduous oaks (*Quercus pubescens*) and one plot dominated by evergreen oaks (*Q. ilex*).

clutches and the peak-date of caterpillar frass fall was confirmed when individual females were followed from one year to the next (data not presented).

**(b) *Aviaries***

The two blue tit populations that nicely matched the peak-date of caterpillar frass fall in broad-leaved deciduous oak woodland bred at the same time in the field, and showed the same inter-year fluctuations in the timing of breeding (see above). We therefore predicted the same timing of breeding for both populations in standardized conditions in captivity. The mainland and Corsica-Muro population started to breed later in captivity than in the field (figure 2), which could be attributed to a habitat effect. Unexpectedly, however, the two captive populations had large and consistent differences in the date of nest construction (mainland: 1 April $\pm$ 13.2 days, $n = 13$, versus Corsica-Muro: 10 May $\pm$ 13.2 days, $n = 19$, $F_{1,30} = 65.3$, $p < 0.0001$) and the onset of egg laying (mainland: 27 April $\pm$ 7.1 days, $n = 13$, versus Corsica-Muro: 28 May $\pm$ 7.4 days, $n = 14$, $F_{1,25} = 129.4$, $p < 0.0001$). The large inter-population differences in timing of breeding were confirmed when analyses controlled for the age at which the birds were put in captivity.

In the field, the Corsica-Pirio birds laid eggs more than one month later than the Corsica-Muro birds (see above). Most surprisingly, the opposite was found in captivity, with highly significantly later nest construction dates (Muro: 10 May $\pm$ 13 days, $n = 19$, versus Pirio: 26 April $\pm$ 9.5 days, $n = 16$, $F_{1,33} = 12.1$, $p < 0.005$) and egg-laying dates (Muro: 27 May $\pm$ 7.7 days, $n = 14$, versus Pirio: 11 May $\pm$ 6.5 days, $n = 17$, $F_{1,29} = 38.0$, $p < 0.0001$) for the Corsica-Muro than for the Corsica-Pirio birds. Thus the observed between-population differences in onset of egg laying in the field completely differed from those obtained with outdoor aviaries (figure 2).

## 4. DISCUSSION

Experiments with captive animals in standardized conditions are used to test if population differences in the
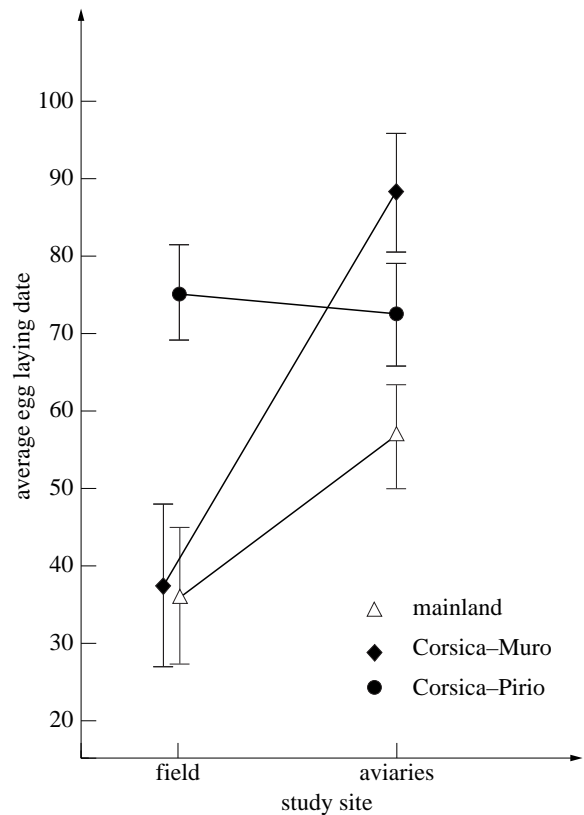
Figure 2. Onset of egg laying in first clutches from wild and captive mainland and Corsican blue tits. Averages and standard deviations are presented. Sample sizes are available in §3.

wild result from genetic differences or phenotypic plasticity (Visser & Lambrechts 1999). If population differences result from phenotypic plasticity, the differences observed in the field should disappear in standardized conditions in captivity. Our study provides the unexpected pattern that large between-population differences exist in captive animals exposed to standardized conditions and that these differences do not exist in the wild, where many confounding variables (food availability, social factors, predation pressures, etc.) can influence phenotypic expression. Although we do not yet know the origin of our unexpected results with captive blue tits, our data exclude several potential explanations concerning between-population differences in sampling, maintenance or physical condition of the captive birds. First, the captive Corsica-Muro blue tits with abnormally late breeding times did not represent a biased sample of the natural population, because the birds in the aviary experiments were trapped during five different trapping sessions in five different years. Furthermore, for two late captive first-year birds from Muro (both constructed, one laid eggs) the parents were known. In both cases, the parents bred more than one month earlier in the field than their captive offspring. One other female laid her eggs more than one month earlier in the field than in captivity. Basic protocols to maintain the birds did not change between years resulting in average lay dates that did not change between study periods (e.g. Pirio 1986–1992: 11 May $\pm$ 7.5 days, $n = 8$, versus 1994–1997: 11 May $\pm$ 6 days, $n = 9$). Although we do not have direct measures of physical condition and energy budgets, we

found that individual breeding performance persisted in different years, e.g. there is a high repeatability of lay dates (e.g. Lambrechts & Dias 1993). Although we may assume that the formation of eggs is energetically much more expensive than the start of nest construction, the large population differences in onset of egg laying were confirmed with the data on the timing of nest construction.

At the landscape level, Corsican blue tits show much more between-population variation in onset of egg laying than mainland blue tits (see §1). Two distinct scenarios can be proposed to explain why breeding times differ so much between the Corsican blue tit populations. A first scenario is that the mainland blue tits are 'programmed' for deciduous and all Corsican blue tits are 'programmed' to discriminate between deciduous and evergreen habitat. This scenario assumes that Corsican blue tits result from a single genetic line that shows much more phenotypic plasticity in response to spatial variation in food and/or tree phenology than the mainland blue tits. This scenario assumes that blue tits use the local food abundance essential to form eggs or the local tree phenology to decide when to start reproduction (e.g. Wingfield 1980; Blondel *et al.* 1993). Here we predict that the Corsica-Pirio and Corsica-Muro blue tits should have the same late breeding time in captivity, a prediction not supported by our data (see above). Furthermore, 'mis-programmed' Corsican blue tit breeding times have been observed in small broad-leaved deciduous woodland plots that are situated in valleys dominated by evergreen woodland (Lambrechts & Dias 1993; Dias & Blondel 1996*a*; M. M. Lambrechts, P. Perret, M. Maistre and J. Blondel, unpublished data).

A second scenario is that the combination of fast local microevolutionary processes in combination with low dispersal on the island of Corsica resulted in different genetic blue tit lines, one line adapted to the early tree phenology in broad-leaved deciduous oak woodland, the other adapted to the late tree phenology in evergreen oak woodland (see Lambrechts *et al.* 1997*a*). Because broad-leaved deciduous oak woodland with early phenology is relatively rare on Corsica, we do not exclude that the special genetic line of Muro is only found in one valley on Corsica, perhaps established by individuals with traits that are not found in the mainland and/or Corsica-Pirio birds. Two potential non-functional explanations can be provided for the abnormally late breeding time of the captive Corsica-Muro blue tits. Perhaps certain cues or resources that are used in the field are missing in captivity and the Corsica-Muro birds are more sensitive to the absence of these cues or resources than the other blue tit populations. Alternatively, blue tits from Muro have more difficulty coping with new and artificial environments than blue tits from the other populations. Different species, populations or individuals may show large differences in their capacity to cope with new environments (Winkler & Leisler 1999). For instance, within local great tit (*Parus major*) populations, large between-individual differences in the exploration of new environments exist (Verbeek *et al.* 1994) and some organisms are 'programmed' to be more shy than others (Wilson *et al.* 1994). This 'psychological' (see Wilson *et al.* 1994) capacity to cope with new environments may act independently of life-history traits if the response mechanisms used in

prospecting new habitats differ from those used in reproductive decision making.

Our case study demonstrates clearly that experiments with captive animals testing the origin of variation in phenotypic traits without knowledge of the ecology of natural populations may lead to erroneous conclusions. For many years long-term studies concluded that Corsican blue tits have late breeding seasons. This conclusion would not have been altered if the studies on Corsica-Muro blue tits were limited to studies with captive individuals. If we accept the possibility that different organisms do not have the same capacity to cope with new and artificial environments (see above), welfare studies taking the natural habits and habitats into account (cf. Dawkins 1998; King 1999) may improve the reliability of experimental work that tests hypotheses in standardized conditions and thus avoid unexpected results that represent artefacts of experimental design. The final conclusion is that results obtained with laboratory experiments using non-domesticated animals should always be verified with population field studies to avoid a misinterpretation of the results obtained.

## REFERENCES

Blondel, J. & Isenmann, P. 1979 Insularité et démographie des mésanges du Genre *Parus* (Aves). *C.R. Acad. Sci. Paris* **289**, 161–164.

Blondel, J., Perret, P. & Maistre, M. 1990 On the genetic basis of the laying date in an island population of blue tits. *J. Evol. Biol.* **3**, 469–475.

Blondel, J., Dervieux, A., Maistre, M. & Perret, P. 1991 Feeding ecology and life history variation of the blue tit in Mediterranean deciduous and sclerophyllous habitats. *Oecologia* **88**, 9–14.

Blondel, J., Dias, P. C., Maistre, M. & Perret, P. 1993 Habitat heterogeneity and life-history variation of Mediterranean blue tits. *Auk* **110**, 511–520.

Blondel, J., Maistre, M., Perret, P., Hurtrez-Boussès, S. & Lambrechts, M. M. 1998 Is the small clutch size of a Corsican blue tit population optimal? *Oecologia* **117**, 80–89.

Dawkins, M. S. 1998 Evolution and animal welfare. *Q. Rev. Biol.* **73**, 305–328.

Dias, P. C. & Blondel, J. 1996*a* Breeding time, food supply and fitness components in Mediterranean blue tits. *Ibis* **138**, 108–113.

Dias, P. C. & Blondel, J. 1996*b* Local specialization and maladaptation in the Mediterranean blue tit (*Parus caeruleus*). *Oecologia* **107**, 79–86.

Dias, P. C., Verheyen, G. R. & Raymond, M. 1996 Source–sink populations in Mediterranean blue tits: evidence using single-locus minisatellite probes. *J. Evol. Biol.* **9**, 965–978.

Gwinner, E. 1996 Circannual clocks in avian reproduction and migration. *Ibis* **138**, 47–63.

Kendeigh, S. C. 1941 Length of day and energy requirements for gonad development and egg laying in birds. *Ecology* **22**, 237–248.

Kern, M. D. 1972 Seasonal changes in the reproductive system of the female white-crowned sparrow, *Zonotrichia leucophrys gambelii*, in captivity and in the field. *Z. Zellforsch.* **126**, 297–319.

King, C. E. 1999 Environmental enrichment and bird welfare. In *Proceedings of the 22nd International Ornithological Congress* (ed. N. J. Adams & R. H. Slotow). Durban. (In the press.)

Kohane, M. J. & Parsons, P. A. 1988 Domestication. Evolutionary change under stress. *Evol. Biol.* **23**, 31–48.

Lambrechts, M. M. & Dias, P. C. 1993 Differences in the onset of egg laying between island and mainland Mediterranean blue tits *Parus caeruleus*: phenotypic plasticity or genetic differences? *Ibis* **135**, 451–455.

Lambrechts, M. M., Perret, P. & Blondel, J. 1996 Adaptive differences in the timing of egg laying between different populations of birds result from variation in photoresponsiveness. *Proc. R. Soc. Lond.* B **263**, 19–22.

Lambrechts, M. M., Blondel, J., Hurtrez-Boussès, S., Maistre, M. & Perret, P. 1997a Adaptive inter-population differences in blue tit life-history traits on Corsica. *Evol. Ecol.* **11**, 599–612.

Lambrechts, M. M., Blondel, J., Maistre, M. & Perret, P. 1997b A single response mechanism is responsible for evolutionary adaptive variation in a bird's laying date. *Proc. Natl Acad. Sci. USA* **94**, 5153–5155.

Meijer, T. & Schwabl, H. 1989 Hormonal patterns in breeding and nonbreeding kestrels, *Falco tinnunculus*: field and laboratory studies. *Gen. Comp. Endocrinol.* **51**, 303–308.

Murton, R. K. & Westwood, N. J. 1977 *Animal breeding cycles*. Oxford: Clarendon.

Pascual, J. A. & Peris, S. J. 1992 Effects of forest spraying with two application rates of cypermethrin on food supply and on breeding success of the blue tit (*Parus caeruleus*). *Environ. Toxicol. Chem.* **11**, 1271–1280.

Perrins, C. M. 1991 Tits and their caterpillar food supply. *Ibis* **133**, 49–54.

SAS Institute Inc. 1989 *SAS/STAT user's guide*, v. 6, 4th edn. Cary, NC: SAS Institute, Inc.

Silverin, B. & Westin, J. 1995 Influence of the opposite sex on photoperiodically induced LH and gonadal cycles in the willow tit (*Parus montanus*). *Horm. Behav.* **29**, 207–215.

Van Noordwijk, A. J., McCleery, R. H. & Perrins, C. M. 1995 Selection for the timing of great tit breeding in relation to caterpillar growth and temperature. *J. Anim. Ecol.* **64**, 451–458.

Verbeek, M. E. M., Drent, P. J. & Wiepkema, P. R. 1994 Consistent individual differences in early exploratory behaviour of male great tits. *Anim. Behav.* **48**, 1113–1121.

Visser, M. E. & Lambrechts, M. M. 1999 Information constraints in the timing of reproduction in temperate zone birds: great and blue tits. In *Proceedings of the 22nd International Ornithological Congress* (ed. N. J. Adams & R. H. Slotow). Durban. (In the press.)

Wilson, D. S., Clark, A. B., Coleman, K. & Dearstyne, T. 1994 Shyness and boldness in humans and other animals. *Trends Ecol. Evol.* **9**, 442–446.

Wingfield, J. C. 1980 Fine temporal adjustment of reproductive functions. In *Avian endocrinology* (ed. A. Epple & M. H. Stetson), pp. 367–390. New York: Academic Press.

Winkler, H. & Leisler, B. 1999 Curiosity in birds: functions and mechanisms. In *Proceedings of the 22nd International Ornithological Congress* (eds. N. J. Adams & R. H. Slotow). Durban. (In the press.)

Zandt, H. 1994 A comparison of three sampling techniques to estimate the population size of caterpillars in trees. *Oecologia* **97**, 399–406.

Zandt, H., Strijkstra, A., Blondel, J. & Van Balen, J. H. 1990 Food in two Mediterranean blue tit populations: do differences in caterpillar availability explain differences in timing of the breeding season. In *Population biology of passerine birds, an integrated approach* (ed. J. Blondel, A. Gosler, J.-D. Lebreton & R. H. McCleery), pp. 145–155. Berlin: Springer.