ORIGINAL ARTICLE

Alexandre Roulin · Pierre Bize · Nathalie Tzaud
Matteo Bianchi · Pierre-Alain Ravussin
Philippe Christe

Oxygen consumption in offspring tawny owls *Strix aluco* is associated with colour morph of foster mother

Abstract In several colour polymorphic species, morphs differ in thermoregulation either because dark and pale surfaces absorb solar radiation to a different extent and/or because morphs differ in key metabolic processes. Morph-specific thermoregulation may potentially account for the observation that differently coloured individuals are frequently not randomly distributed among habitats, and differ in many respects, including behaviour, morphology, survival and reproductive success. In a wild population of the colour polymorphic tawny owl *Strix aluco*, a recent cross-fostering experiment showed that offspring raised and born from red mothers were heavier than those from grey mothers. In the present study, we tested in the same individuals whether these morph-specific offspring growth patterns were associated with a difference in metabolic rate between offspring of red and grey mothers. For this purpose, we measured nestling oxygen consumption under two different temperatures (laboratory measurements: 4 and 20°C), and examined the relationships between these data sets and the colour morph of foster and biological mothers. After controlling for nestling body mass, oxygen consumption at 20°C was greater in foster offspring raised by grey foster mothers. No relationship was found between nestling oxygen consumption and coloration of their biological mother. Therefore, our study indicates that in our experiment offspring raised by grey foster mothers showed not only a lower body mass than offspring raised by red foster mothers, but also consumed more oxygen under warm temperature. This further indicates that rearing conditions in nests of grey mothers were more stressful than in nests of red mothers.

Keywords Colour polymorphism · Metabolism · Oxygen consumption · Tawny owl · Temperature

Introduction

The evolution and maintenance of alternative genetically inherited colour morphs in animal and plant populations have fascinated evolutionary ecologists for a long time. Studies performed in molluscs (Jones et al. 1977; Goodhart 1987), reptiles (Andrén and Nilson 1981), anurans (Hoffman and Blouin 2000), insects (Wilson et al. 2001), birds (Roulin 2004) and plants (Armbruster 2002) showed that many genetic colour polymorphisms are not selectively neutral with respect to life history components, since alternative morphs are frequently observed to achieve a different reproductive success. This can often be explained by non-random habitat distribution of individuals with respect to colour morphs (Roulin 2004), suggesting that morphs are under disruptive selection by being adapted to alternative ecological niches. These morph-dependent adaptations may be the outcome of several processes one of which being associated with thermo-regulation.

A large number of colour polymorphisms are due to inter-individual variation in the deposition of melanins, pigments that are under strong genetic control, and are known to play a role in solar radiation (e. g. Berry and Willmer 1986), dark surfaces absorbing and radiating heat more quickly than pale surfaces (Majerus 1998). If coloration can itself play a key role in thermoregulation

A. Roulin (✉) · P. Bize · N. Tzaud · M. Bianchi · P. Christe
Department of Ecology and Evolution, University of Lausanne,
Biology Building, 1015 Lausanne, Switzerland
E-mail: alexandre.roulin@unil.ch
Tel.: +41-21-6924189
Fax: +41-21-6924165

P. Bize
Zoology institute, Division of Evolutionary Ecology,
University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland

P.-A. Ravussin
Rue de Theu, 1446 Baulmes, Switzerland

in ectotherms (e.g. Heath 1975; Gibson and Falls 1979; Bittner et al. 2002; Pereboom and Biesmeijer 2003) and endotherms (Ellis 1980; Wunderle 1981; but see Beasley and Ankney 1988), morphs may also differ with respect to physiological processes associated with thermoregulation. For example, in snails, morph-dependent thermoregulation is not only due to physical differences in shell reflectivity and absorption of solar energy, and to variations in the activity and habitat selection by the various genotypes (e.g. Heath 1975; Jones 1982), it can also be due to neuromodulatory mechanisms (Kavaliers 1992; Sokolova and Berger 2000). This suggests that genes creating variation in coloration pleiotropically affect key physiological processes involved in thermoregulation (Forsman et al. 2002).

Colour polymorphism is particularly frequent in owls with individuals belonging to a same population, sex and age class displaying rufous, brown or grey morphs (König et al. 1999). Some authors have suggested that the performance of alternative morphs is associated with climatic variables. In the screech (*Otus asio*) and tawny (*Strix aluco*) owls, the proportion of red individuals decreased after a harsh winter (Van Camp and Henny 1975; Galeotti and Cesaris 1996), maybe because rufous and grey individuals differ in their ability to thermoregulate (Mosher and Henny 1976). Morph-dependent thermo-regulation may explain why, in a cross-fostering experiment, foster nestlings were heavier when their foster and biological mothers were redder (Roulin et al. 2004). Perhaps environmental conditions prevailing during this experiment (carried out in a wild population in 2003) were particularly favourable to the red morph, with chicks of red foster and biological mothers showing better thermo-regulation activities than those of grey mothers.

In the present study, we tested a prediction of this hypothesis by measuring oxygen consumption in the laboratory in some of the same cross-fostered nestlings used in Roulin et al. (2004). Based on the fact that stressful environmental conditions may lead to an elevation of oxygen consumption (Demas et al. 1997; Svensson et al. 1998; Ots et al. 2001; Giorgi et al. 2001; Moreno et al. 2001; Raberg et al. 2002), we predict that chicks of red foster and/or biological mothers have lower oxygen consumption than chicks of grey foster and/or biological mothers. Our aim is therefore to investigate whether the covariation between offspring body condition and mother colour morph found in Roulin et al. (2004) is associated with a covariation between offspring oxygen consumption and mother colour morph. Two non-mutually exclusive scenarios are possible. First, if genes creating variation in coloration pleiotropically affect key physiological processes involved in thermoregulation, offspring oxygen consumption is expected to covary with the coloration of biological mothers. Second, if environmental conditions prevailing in this experiment were favourable to parents of a given morph, offspring oxygen consumption is expected to covary with the coloration of foster mothers.

Methods

The study organism

The tawny owl is sexually dimorphic in size, with adult females weighing on average 562 g and males 437 g (Baudvin and Dessolin 1992). Adult birds, but not nestlings, can be easily classified in colour morphs with coloration varying between red and grey. The proportion of each morph is similar in the two sexes (Baudvin and Dessolin 1992; Galeotti and Cesaris 1996). Apparently, pairing is random with respect to plumage coloration (Roulin et al. 2003). Birds mainly breed in holes of trees located in forests, and the diet is primarily composed of small mammals and complemented by birds and frogs. Nestlings abandon their nests before being able to fly at approximately 4 weeks of age.

Experimental design

The study was carried out in 2003 in western Switzerland. We matched 18 nests in pairs with similar laying dates (Pearson correlation: $r = 0.95$, $n = 9$, $P = 0.0001$) and clutch sizes ($r = 0.71$, $n = 9$, $P = 0.033$). During incubation, we captured all females (males were absent from the nest at that time) and Roulin classified them in one of five colour morphs (1 = red, 2 = red-brown, 3 = brown, 4 = brown-grey and 5 = grey). One week later, we recaptured 16 of them for an independent classification into colour morphs. The two measurements were repeatable (one-way ANOVA, repeatability = 0.87, $F_{17,16} = 14.98$, $P < 0.0001$) demonstrating the reliability of our scoring system (see also Roulin et al. 2003). In total, we had a sample of two red females, six red-brown, four brown, four brown-grey and two grey females. We swapped all eggs or recently hatched chicks between pairs of nests, so that each pair raised alien young, to determine whether metabolic rate is associated with colour morph of foster and biological mothers. Within pairs of nests foster and biological mothers did not resemble each other with respect to colour morph ($r = -0.13$, $n = 9$, $P = 0.75$). Hatching date (mean 6 April, range 21 March–25 April), clutch size (mean 3.4, range 2–5) and altitude (mean 672, range 470–870) were not correlated with female colour morph (Pearson correlation, P-values > 0.30). Number of hatchlings ($r = 0.40$, $n = 18$, $P = 0.10$) and proportion of male offspring (i.e. sex ratio, $r = -0.04$, $n = 17$, $P = 0.87$) were not significantly associated with colour morph of the foster mother

When nestlings were 25 days of age (range 18–30), we randomly selected one individual per brood to measure oxygen consumption. We chose this age because feathers are well grown and nestlings are already thermo-independent. Nestling body mass ranged between 240 and 412 g (mean 330 g). For unknown reasons one nest was abandoned, and because of technical problems we were unable to measure oxygen consumption of chicks from one of the nest. Therefore, sample size is 16 experimental

nestlings. For each nestling, a drop of blood was collected from the brachial vein, and their sex determined from blood cell DNA using polymerase chain reaction (PCR) amplification of the CHD genes (Griffiths et al. 1998).

Assessment of oxygen consumption

Oxygen consumption (VO^2) was measured using an open-air flow respirometer. Owls were placed in a metabolic chamber ($40\times40\times40$ cm^3 = 64 l) that was located in a climatic chamber (Weiss Technik, Germany) to calibrate temperature. There was no light in the chamber during the tests. Outside air was dried over silica gel and pushed through the metabolic chamber at a flow rate of ca. 75 l/h. The flow rate was controlled and measured continuously by a calibrated mass flow controller (Model 5850E, Brooks Instruments, Veenendaal, The Netherlands) that was connected to a control and read out equipment (Model 5878, Brooks Instruments). The effluent air was sequentially passed through a column of KOH (to fix the expired CO_2) and a silica gel column (to fix H_2O). Finally, oxygen concentration was measured using an oxygen analyser (Gas purity analyser Xentra 4100, Servomex, Esslinger, Switzerland). It was recorded on paper by a potentiometric recorder (recorder 320, Scientific Instruments, Basel, Switzerland) and digitised using the software BioBench (Version 1.0, National Instruments). We calculated oxygen consumption with the Depocas and Hart's formula (1957): $VO_2 = V_2 \times (F_1O_2 - F_2O_2)/(1 - F_1O_2)$. Under our experimental conditions, the Depocas and Hart's formula is equal to $1.2647 \times V_2 \times dFO_2$ with VO_2 being the amount of O_2 consumed by an owl (ml O_2/h), dFO_2 the difference in O_2 measured before and after the metabolic chamber (‰ O_2) and V_2 the flow rate (l/h) measured before the metabolic chamber. Although the apparatus measured dFO_2 200 times/s, only one value was available every 10.5 ± 2.5 s.

The tawny owl is a nocturnal bird, and hence we decided to measure oxygen consumption during the day when the individuals rest. In this way, we reduced the risk of confounding morph-specific metabolic rate with morph-specific behaviour. The apparatus was at the university of Lausanne, and to measure oxygen consumption we brought owlets by car at that place. The distance between nest-boxes and the university was not correlated with colour morph of foster and biological mothers (mean 31 km, range 8–56 km; Pearson correlation, P-values > 0.75). Because morph-dependent thermo-regulation may differ between temperatures, we decided to measure oxygen consumption under two different temperatures, namely 4 and 20°C. The temperatures we chose lay within the natural range, since in our study area the minimal and maximal mean ambient temperatures measured from 21 March to 25 May were 0 and 20.4°C (data collected in Payerne between 1990 and 2003 by the Swiss Meteorological Institute).

Each individual was placed in the chamber at 0800 hours. At that time, the climatic chamber was set up at 1°C, but due to the presence of an owl in the metabolic chamber, actual temperature was 4.3 ± 0.4°C (mean $\pm$ SD; treatment 'cold temperature'). Each individual was placed under this temperature for 4 h 30 min and during that time we measured oxygen consumption. Then, we removed the owl from the chamber during 1 h 30 min, a period of time that was necessary to set up temperature to 18°C. In the meantime, owls were placed in a quiet box. Due to the presence of an owl, temperature in the chamber was 20.0 ± 1.4°C (treatment 'warm temperature'). We measured oxygen consumption under 20°C during 4 h 30 min. We always started with the 4°C treatment and then the 20°C treatment because it was easier to quickly set up the chamber from 4 to 20°C than from 20 to 4°C.

Owls needed time to adapt to the temperatures set up in the metabolic chamber, and hence we did not consider oxygen consumption measured during the first 2 h of each treatment. The amount of oxygen consumed per hour has therefore been calculated for a period of 2 h and 30 min. To derive the minimum amount of oxygen consumed per hour, we considered the minimal value for each 20-min period, and calculated a mean value over the seven available measurements. This measure is not sensitive to variation in physical activities of the owls during the measurements.

Each individual tested in the metabolic chamber has been weighed before (i.e. at 0800 hours) and after the experiment (i.e. at 1830 hours). We also weighed any faeces and pellets produced during the day. For each individual, we calculated a mean body mass value as (body mass at 0800 hours + body mass at 1830 hours + mass of faeces and pellets)/2. All chicks were brought back to their nest between 1900 and 2000 hours. They all fledged successfully.

Statistical procedure

All statistical analyses were carried out with the JMP software (Sall and Lehman 1996). They are two-tailed and P-values < 0.05 considered significant. Minimum oxygen consumption at 4°C was introduced in stepwise ANCOVA using the BACKWARD option with the probability of leaving a factor being 0.10. The independent factors were nestling sex, age, mean body mass and colour morph of foster and biological mothers. We did not consider the colour morph of individual chicks because at the nestling age it is not yet developed, and furthermore the aim of our study was to examine whether offspring oxygen consumption covaries with colour morph of foster and biological mothers. A similar analysis was carried out for minimum oxygen consumption at 20°C. Means are quoted ± 1 SD.

Results

In 25-day-old nestling tawny owls, minimum oxygen consumption at 4°C was 6.24 ± 0.74 l/h and at 20°C

4.38 ± 0.44 l/h $(t_{15} = 12.33, \ P < 0.0001)$. Oxygen consumption measured at 4°C was positively correlated with the consumption at 20°C $(r = 0.58, \ n = 16, \ P = 0.019)$. In stepwise ANCOVA, minimum oxygen consumption at 4°C was significantly associated with nestling age $(F_{1,13} = 4.82, \ P = 0.047, \ \text{standardised } \beta = 0.47)$ but not with nestling body mass $(F_{1,13} = 4.21, \ P = 0.06, \ \beta = 0.44)$, sex $(F_{1,13} = 0.01, \ P = 0.91)$, and colour morph of foster $(F_{1,13} = 1.92, \ P = 0.19)$ and biological mothers $(F_{1,13} = 0.70, \ P = 0.42)$. Minimum oxygen consumption at 20°C was significantly associated with colour morph of foster mother $(F_{1,13} = 6.14, \ P = 0.028, \ \beta = 0.51;$ Fig. 1) and nestling body mass $(F_{1,13} = 13.06, \ P = 0.003, \ \beta = 0.74)$, but not with nestling age $(F_{1,13} = 0.33, \ P = 0.58)$, sex $(F_{1,13} = 0.09, \ P = 0.77)$ and colour morph of biological mother $(F_{1,13} = 0.59, \ P = 0.46)$.

Discussion

In the tawny owl, a cross-fostering experiment showed that redder biological and foster mothers produced heavier offspring (Roulin et al. 2004). In a first move to understand the proximate mechanisms explaining these findings, we analysed oxygen consumption in the same individuals as in Roulin et al. (2004) with the aim of determining whether metabolic rate is associated with colour morph of foster and/or biological mothers. One individual was tested per nest and we found that when the foster mother was greyer foster chicks consumed more oxygen at least under 20°C. This suggests that, in 2003, rearing conditions experienced in nests of grey
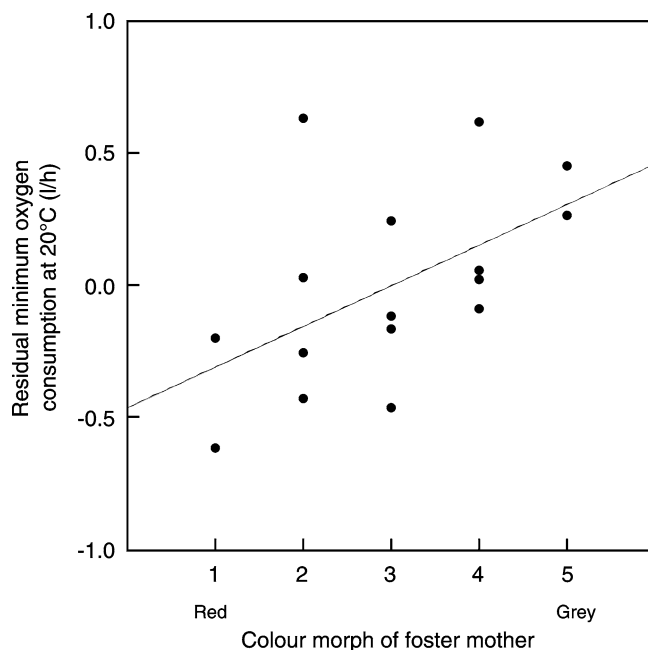

Fig. 1 Relationship between residual minimum oxygen consumption at 20°C and colour morph of foster mother tawny owls (*Strix aluco*). Residuals were extracted from a regression analysis with minimum oxygen consumption as the dependent variable and nestling body mass as the independent variable

mothers were not optimal, leading foster chicks to grow to a lower body mass and to have a higher metabolic rate under standardised conditions. Complementary studies are required to determine which environmental factors caused these observations (e.g. quality and/or quantity of food, parasites). The absence of a relationship between offspring oxygen consumption and colour morph of biological mother under both 4 and 20°C is difficult to interpret because sample size was low and our design did not allow to partition the origin-related from environmental components of oxygen consumption. To gain more confidence on whether offspring metabolism is associated with colour morph of biological mother, we should measure several chicks born from different mothers and raised in the same nest (partial cross-fostering experiment). We can indeed suspect that metabolism covaries with colour polymorphism if genes coding for coloration pleiotropically alter key physiological processes. The tawny owl therefore appears to be an appropriate model system to examine the full range of adaptations of genetically inherited morphs in an ecological context.

Zusammenfassung

Der Sauerstoffverbrauch bei jungen Waldkäuzen *Strix aluco* hängt von der Farbmorphe der Pflegemutter ab

Bei verschiedenen farbpolymorphen Arten unterscheiden sich die Morphen in ihrer Thermoregulation entweder, weil dunkle und helle Oberflächen Sonnenstrahlung in unterschiedlichem Ausmaß absorbieren, und/oder, weil sich die Morphe in wesentlichen Stoffwechselprozessen unterscheiden. Morphespezifische Thermoregulation könnte möglicherweise die Beobachtung begründen, dass farbunterschiedliche Individuen häufig nicht zufällig in den Habitaten verteilt sind und sich in vielem, darunter Überlebens- und Fortpflanzungserfolg, Verhalten und Morphologie unterscheiden. Bei einer wildlebenden Population des farbpolymorphen Waldkauz *Strix aluco* zeigte ein kürzlich durchgeführtes Experiment, bei dem Jungtiere von Müttern der jeweils anderen Farbmorphe aufgezogen wurden, dass von roten Muttertieren abstammende und aufgezogene Jungtiere schwerer waren als jene von grauen Muttertieren. In der vorliegenden Studie testeten wir an denselben Individuen wie bei, ob diese morphspezifischen Wachstumsmuster der Jungtiere mit einem Unterschied in der Stoffwechselrate zwischen Nachkommen von roten und grauen Müttern zusammenhing. Zu diesem Zweck maßen wir den Sauerstoffverbrauch der Jungtiere bei zwei verschiedenen Temperaturen (4 und 20°C) und untersuchten den Zusammenhang zwischen Sauerstoffverbrauch und Farbmorphe von Pflege- und biologischer Mutter. Es wurde kein Zusammenhang gefunden zwischen dem Sauerstoffverbrauch und der Farbe der biologischen Mutter. Dagegen zeigte sich, dass von grauen Pflegemüttern

aufgezogene Jungtiere nicht nur eine geringere Körpermasse hatten als von roten Pflegemüttern aufgezogene Tiere, sondern auch mehr Sauerstoff bei warmen Temperaturen verbrauchten. Das weist darauf hin, dass die.

References

Andrén C, Nilson G (1981) Reproductive success and risk of predation in normal and melanistic colour morphs of the adder, *Vipera berus*. Biol J Linn Soc 15:235–246

Armbruster WS (2002) Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera. J Evol Biol 15:468–486

Baudvin H, Dessolin JL (1992) Analyse de la morphométrie de la chouette hulotte *Strix aluco* en Bourgogne. Alauda 60:93–104

Beasley BA, Ankney CD (1988) The effect of plumage color on the thermoregulation abilities of lesser snow goose goslings. Can J Zool 66:1352–1358

Beck B (2000) Neuropeptides and obesity. Nutrition 16:916–923

Berry AJ, Willmer PG (1986) Temperature and the colour polymorphism of *Philaenus spumarius* (Homoptera: Aphrophoridae). Ecol Entomol 11:251–259

Bittner TD, King RB, Kerfin JM (2002) Effects of body size and melanism on the thermal biology of garter snakes (*Thamnophis sirtalis*). Copeia 2:477–482

Demas GE, Chefer V, Tala MI, Nelson RJ (1997) Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. Am J Physiol 273:1631–1637

Depocas F, Hart SJ (1957) Use of the Pauling oxygen consumption of animals in open-circuit systems and in short-lag, closed-circuit apparatus. J Appl Physiol 10:388–392

Ellis HI (1980) Metabolism and solar radiation in dark and white herons in hot climates. Physiol Zool 53:358–372

Forsman A, Ringblom K, Civantos E, Ahnesjö J (2002) Coevolution of color pattern and thermoregulatory behavior in polymorphic pygmy grasshoppers *Tetrix undulata*. Evolution 56:349–360

Galeotti P, Cesaris C (1996) Rufous and grey colour morphs in the Italian tawny owl: geographical and environmental influences. J Avian Biol 27:15–20

Gibson AR, Falls JB (1979) Thermal biology of the common garter snake *Thamnophis sirtalis* (L.). II. The effects of melanism. Oecologia 43:97–109

Giorgi MS, Arlettaz R, Christe P, Vogel P (2001) The energetic grooming costs imposted by a parasitic mite (*Spinturnix myoti*) upon its bat host (*Myotis myotis*). Proc R Soc Lond B 268:2071–2075

Goodhart CB (1987) Why are some snails visibly polymorphic, and others not? Biol J Linn Soc 31:35–58

Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. Mol Ecol 7:1071–1075

Heath DJ (1975) Colour, sunlight and internal temperatures in the land-snail *Cepaea nemoralis* (L.). Oecologia 19:29–38

Hoffman EA, Blouin MS (2000) A review of colour and pattern polymorphisms in anurans. Biol J Linn Soc 70:633–665

Jones JS (1982) Genetic differences in individual behaviour associated with shell polymorphism in the snail *Cepaea nemoralis*. Nature 298:749–750

Jones JS, Leith BH, Rawlings P (1977) Polymorphism in *Cepaea*: a problem with too many solutions? Annu Rev Ecol Syst 8:109–143

Kavalier M (1992) Opioid systems, behavioral thermoregulation and shell polymorphism in the land snail, *Cepaea nemoralis*. J Comp Physiol B162:172–178

König C, Weick F, Becking J-H (1999) A guide to the owls of the world. Pica, East Sussex, UK

Majerus MEN (1998) Melanism, evolution in action. Oxford University Press, Oxford

Moreno J, Sanz JJ, Merino S, Arriero E (2001) Daily energy expenditure and cell-mediated immunity in pied flycatchers while feeding nestlings: interaction with moult. Oecologia 129:492–497

Mosher JA, Henny CJ (1976) Thermal adaptiveness of plumage color in screech owls. Auk 93:614–619

Ots I, Kerimov AB, Ivankina EV, Ilyina TA, Horak P (2001) Immune challenge affects basal metabolic activity in wintering great tits. Proc R Soc Lond B 268:1175–1181

Pereboom JJM, Biesmeijer JC (2003) Thermal constraints for stingless bee foragers: the importance of body size and coloration. Oecologia 137:42–50

Raberg L, Vestberg M, Hasselquist D, Holmdahl R, Svensson E, Nilsson JA (2002) Basal metabolic rate and the evolution of the adaptive immune system. Proc R Soc Lond B 269:817–821

Roulin A (2004) The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. Biol Rev 79:815–848

Roulin A, Ducret B, Ravussin P-A, Altwegg R (2003) Female plumage coloration covaries with reproductive strategies in the tawny owl. J Avian Biol 34:393–401

Roulin A, Bize P, Ravussin P-A, Broch L (2004) Genetic and environmental effects on the covariation between colour polymorphism and a life history trait. Evol Ecol Res 6:1253–1260

Sall J, Lehman A (1996) JMP start statistics. A guide to statistical and data analysis using JMP and LMP in software. Duxbury Press, Wadsworth, London

Sokolova IM, Berger VJ (2000) Physiological variation related to shell colour polymorphism in White Sea *Littorina saxatilis*. J Exp Mar Biol Ecol 245:1–23

Svensson E, Raberg L, Koch C, Hasselquist D (1998) Energetic stress, immunosuppression and the costs of an antibody response. Funct Ecol 12:912–919

Taylor AW, Namba K (2001) In vitro induction of CD25$^+$ CD4$^+$ regulatory T cells by the neuropeptide alpha-melanocyte stimulating hormone. Immun Cell Biol 79:358–367

Van Camp LF, Henny CJ (1975) The screech owl: its life history and population ecology in northern Ohio, North American Fauna 71. U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C.

Wilson K, Cotter SC, Reeson AF, Pell JK (2001) Melanism and disease resistance in insects. Ecol Lett 4:637–649

Wunderle JM (1981) An analysis of a morph ratio cline in the bananaquit (*Coereba flaveola*) on Grenada, West Indies. Evolution 35:333–344