Physiological ecology of incubation of ptarmigan eggs at high and low altitudes

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Physiological ecology of incubation of ptarmigan eggs at high and low altitudes

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The broad altitudinal distribution of ptarmigan *Lagopus* spp. makes this group an excellent model with which to ask how morphological and physiological specializations foster successful reproduction of birds at their distributional limits. Eggshell conductance and permeability to gases, shell surface area, shell thickness, daily water loss, and egg volume were measured in willow ptarmigan *Lagopus lagopus* breeding at 720 m a.s.l. and white-tailed ptarmigan *Lagopus leucurus* breeding between 3,600 and 4,100 m a.s.l. Of these characteristics, only egg volume and shell surface area differed significantly between the two species. Since the rate of daily water loss is similar at the two breeding locations, embryos develop in similar hydric environments inside the shell. High altitude embryos facilitate oxygen delivery with acclimatory adjustments in certain cardiovascular and cellular characteristics.

Key words: egg mass, eggshell conductance, high altitude, incubation, water loss, white-tailed ptarmigan, willow ptarmigan

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Birds are among the most successful vertebrates in terms of their ability to breed over broad geographical gradients. For this reason, they provide an excellent model for examining how animals respond to variations in the physical environment over geographical gradients and to what extent survival at the edges of geographical distributions depends on physiological acclimatization or genetic changes.

Barometric pressure (P_B, in torr) is a physical feature of the environment that varies continuously with increasing altitude. Increased cold temperatures, intensity of solar radiation, hypoxia (low oxygen partial pressures), and decreased water vapour pressure occur as secondary consequences of the reduction in P_B at high altitudes. Some of these factors, such as cold and hypoxia, may act synergistically to pose even greater challenges to animal existence than either factor acting alone. Despite these problems, at least 20 species of birds breed successfully above 4,000 m; the record altitude for nesting is around 6,500 m (Rahn 1977, Carey, Leon-Velarde & Monge 1991 and references therein). A few species breed over the entire altitudinal gradient from sea level to above 4,000 m, but most species breeding above 4,000 m do not breed at lower altitudes (Parker, Parker & Plenge 1982, Inskip & Inskip 1985)

Ptarmigan *Lagopus* spp. have a broad geographical and elevational distribution. Details about geographic variation in their breeding biology have been summarized by Braun, Martin & Robb (1993) and Martin, Holt & Thomas (1993). Two species within this genus were chosen for study because embryonic precocial development in relatively large eggs facilitates measurements of a variety of characteristics of both shell and embryo. This paper compares features of eggshells which govern gas exchange. We will also summarize other research concerning whether physiological differences between lowland and montane ptarmigan embryos result from physiological acclimatization to hypoxia or from genetic adaptations.

General background

The ability of birds to breed over broad geographical gradients depends, in part, on their ability to meet the requirements of the embryos for development within their tolerance limits. Since all the nutrients and water necessary for the growth and maintenance of the embryo until hatching are prepackaged in the egg before laying (review by Carey 1996), the principal physical requirements of the embryos are heat and gas exchange. To hatch successfully, embryos must receive sufficient heat, usually, but not always (Howell 1979, Seymour & Ackerman 1980), from an incubating parent. The interrelation between incubation constancy and ambient temperatures in the biology of ptarmigan reproduction is reviewed by Wiebe & Martin (1997).

Embryos also have a mandatory requirement for appropriate levels of gas exchange between the interior of the egg and the environment. Oxygen flows into the egg and CO_2 and water vapour move out of the egg principally by the process of diffusion down concentration gradients. The gradients for O_2 and CO_2 are established by the metabolism of the embryo and the gradient for water vapour is set by the differential between the fully saturated atmosphere of the egg and the (usually) less saturated environment of the nest (Wangensteen & Rahn 1970/71). The factors that govern the rate of diffusion of a gas through the shell ($\dot{M}$, cm^3 STPD) have been described by a modification of the Fick equation (Paganelli, Ar, Rahn & Wangensteen 1975):

$$\dot{M} = (D/RT) \cdot (Ap/L) \cdot \Delta P \qquad (1)$$

where D = binary diffusion coefficient ($cm^2 \cdot sec^{-1}$), RT = gas constant and absolute temperature (cm^3 STPD $\cdot\, cm^{-3} \cdot torr^{-1}$), L = length of the diffusion path or shell thickness (cm), Ap = effective pore area (cm^2), and ΔP = partial pressure difference of gas across the shell (torr). Of all the factors in this equation, the female has control over only shell thickness and pore area. The factors (D/RT) · (Ap/L) are often combined into a single value 'G', the conductance or diffusive capacity of the egg to a gas ($cm^3 \cdot sec^{-1} \cdot torr$) (Ar, Paganelli, Reeves, Greene & Rahn 1974). Therefore, equation 1 becomes:

$$\dot{M} = G \cdot \Delta P \qquad (2)$$

Conductance measurements are typically standardized to 760 torr and 25°C to facilitate comparisons of eggshell conductance of various species collected in a variety of locations. Conductance of the egg to water vapour (GH_20) is typically measured with an easy and accurate method (Ar et al. 1974); conductance to O_2 and CO_2 can be calculated from the value for water vapour (Paganelli, Ackerman & Rahn 1978).

The average eggshell conductance of a species is thought to be under selection from competing requirements (Paganelli et al. 1978). Since O_2 diffuses in the opposite direction from that of CO_2 and water vapour, the conductance must be large enough to allow sufficient O_2 to diffuse in, yet small enough to prevent excessive losses of CO_2 and water vapour. Therefore, the average G of a species represents a compromise between these mutually antagonistic factors. Shell conductance regulates both the rate of gas exchange and prepares the embryo for the onset of convective respiration (Wangensteen & Rahn 1970/71). If conductance is too high or too low for a given set of incubation conditions, the embryo may fail to hatch (Wangensteen, Wilson & Rahn 1970/71, Rahn, Paganelli & Ar 1974, Ar & Rahn, 1980). A review of the data underlying these principles is presented by Carey (1983).

Problems of gas exchange at high altitude

The diffusion coefficient (D) in equation 1 is inversely proportional to barometric pressure (Rahn et al. 1974). Therefore, gas diffusion will increase at altitude if all other factors in equation 1 are held equal (Paganelli et al. 1975). The effective G of an egg increases with altitude proportionally with the decrease in barometric pressure. If an egg were laid at an altitude at which the P_B were half (0.5 atm) the value at sea level, gases would diffuse twice as fast through the shell (Paganelli et al. 1975). This effect would increase the rate of loss of CO_2 and water vapour from the egg and also would increase the rate of O_2 diffusion into the egg. The latter effect would benefit the embryo by offsetting, to a certain extent, the decrease in partial pressure of O_2 at low barомetric pressure (Visschedijk, Ar, Rahn & Piiper 1980). However, increased losses of water vapour and CO_2 could be fatal to an embryo. If such variation in gas exchange was detrimental to montane embryos in the evolution of birds breeding at high altitude, what mechanisms might have developed to ameliorate this situation?

Previous studies on conspecific and congeneric birds breeding between sea level and 4,478 m a.s.l. have indicated that eggshell conductance to gases (corrected to 760 torr) is reduced in approximate proportion to the reduction in P_B (and increase in diffusion coefficients for gases) at each breeding location between sea level and about 3,600 m a.s.l. (Packard, Sotherland & Packard 1977, Rahn, Carey, Balmas, Bhatia & Paganelli 1977, Carey, Garber, Thompson & James 1983). Since the reduction in G roughly corresponds to the increase in D, losses of water vapour and (presumably) CO_2 are independent of altitude (Carey et al. 1983). This trend of reduction in G is reversed at altitudes above 3,600 m: the reduction in eggshell conductance does not compensate for the decrease in P_B and in some species, G is near or even above sea level values (Carey, Leon-Velarde, Dunin-Borkowski, Bucher, de la Torre, Espinosa & Monge 1989a, Carey, Leon-Velarde, Dunin-Borkowski & Monge 1989b, Carey et al. 1991). Variation in mean eggshell conductance is achieved in all species studied thus far except one (Puna ibis *Plegadis ridgwayi*; Carey, Leon-Velarde, Castro & Monge 1987) by a reduction in the numbers of pores, rather than in the sizes of pores or shell thickness (Carey et al. 1989a,b). This curvilinear relation between eggshell conductance and P_B is thought to reflect changing priorities for gas exchange: That is, conservation of water vapour and CO_2 at the expense of O_2 availability to the embryo up to about 3,600 m a.s.l., and then at higher altitudes, selection for improvement of O_2 diffusion occurs at the expense of increased losses of water vapour and CO_2. Adjustments in shell structure at moderate altitudes serve to maintain a hydric and acid-base environment inside the egg similar to that at sea level, but the interior of the egg becomes progressively hypoxic (Carey, Thompson, Vleck & James 1982). At altitudes above 3,600 m, no possible modification of shell structure can create gaseous conditions similar to those at sea level. Therefore, the physiological properties of the embryo must become progressively more important for fostering growth and development in the abnormal gaseous conditions found inside the egg (hypoxia, hypocapnia, and dehydration) above 4,000 m (Carey et al. 1989a,b, Carey, Dunin-Borkowski, Leon-Velarde, Espinosa & Monge 1993).

Ptarmigan at high and low altitudes

The broad geographic and altitudinal distribution of ptarmigan make this group an appropriate model to address questions concerning how morphological and physiological specializations foster embryonic survival at distributional limits. The questions addressed in our research program were: 1) how do morphological characteristics of eggs and physiological features of embryos of white-tailed ptarmigan *Lagopus leucurus* breeding near their altitudinal limit between 3,600 and 4,100 m a.s.l. differ from those of willow ptarmigan *Lagopus lagopus* breeding at 720 m a.s.l. and, 2) do physiological differences result from acclimatization to environmental factors or from genetically fixed adaptations? This paper will present data on eggshell characteristics and will provide a brief summary of embryonic physiological characteristics in the discussion.

Methods

Willow ptarmigan eggs were collected within 2 km of the Toolik Field Station of the University of Alaska, located north of the Brooks Range at 720 m altitude, 68°38'N, 149°38'W, in Alaska. White-tailed ptarmigan eggs were collected at altitudes of 3,600-4,100 m at Mt. Evans and near Guanella Pass (39°34-40'N, 105°35-53'W), Clear Creek County, Colorado. Data on egg characteristics were taken from only one egg per nest for calculation of group means because some features of avian eggs are more similar within a clutch than compared between clutches of different females (Sotherland, Packard & Taigen 1979). Eggs were wrapped individually in plastic and stored briefly in a refrigerator either at the Toolik Field Station or at the University of Colorado in Boulder, Colorado at 1,600 m a.s.l.

The conductance to water vapour (GH_20) was measured at 25°C according to the method of Ar et al. (1974) and corrected to 760 torr (see Carey et al. 1983 for complete description). Shell thickness without shell membranes was measured with micrometer calipers accurate to 0.025 mm. Egg length and width were used to calculate egg volume, shell surface area, and permeability using the following equations:

$V = 0.51\ LB^2$, (Hoyt 1979) (3)

$SA = 4.94\ V^{0.667}$ (Hoyt 1976) (4)

$KH_20 = GH_20\ /\ SA \cdot 69465.5$ (Rahn et al. 1977) (5)

where V = egg volume in cm^3, L = egg length in cm, B = maximal diameter of egg in cm, SA = surface area in cm^2, KH_2O = eggshell permeability to water vapour in cm^3 STP $\cdot$ $sec^{-1} \cdot cm^{-2} \cdot torr^{-1} \cdot 10^{-6}$, GH_2O = eggshell conductance to water vapour in mg $\cdot$ $day^{-1} \cdot torr^{-1}$ and 69,465.5 = a correction factor used to adjust the units of water vapour from mass to volume and the time units from days to seconds.

Daily water loss of eggs that had been incubated at least five days in the field (see Carey 1983 for effects of age on conductance) was measured by numbering two randomly-selected eggs per clutch with waterproof ink and weighing each to the nearest 0.01 g on a battery-operated Acculab top-loading balance, accurate to 0.0001 g. They were reweighed approximately 24 hours later, the difference in mass representing the loss of water vapour from the egg (Ar & Rahn 1980). The value from the first egg weighed was used to represent the water loss from eggs in that clutch unless it proved to be excessive due to a crack in the shell. In that event, the measurement from the second egg was used.

Results

Egg volume and shell surface area of willow ptarmigan were significantly larger than those of white-tailed ptarmigan (Table 1). However, shell thickness did not vary significantly among the two groups. Eggshell conductance to water vapour (GH_2O), shell permeability (KH_20), and daily water loss in the field of white-tailed and willow ptarmigan were statistically indistinguishable (see Table 1).

Discussion

A large number of characteristics, e.g. incubation period, water loss, conductance to water vapour, surface area, shell thickness, pore area, egg volume, pores per egg, vary as a function of fresh egg mass in birds (see review by Carey 1983). Fresh egg mass of willow ptarmigan averages about 22.4 g (K. Martin, unpubl. data). Unfortunately, no data are available on fresh egg mass of white-tailed ptarmigan. The significant differences in egg volume and surface area found in this study undoubtedly reflects a difference in average egg mass of the two species.

Despite this difference in egg size (volume), neither GH_2O nor eggshell permeability corrected for surface area (KH_2O) varied significantly between these two species. When data from a large number of avian species with a broad range of egg masses are considered, conductance to water vapour (GH_2O, in mg $\cdot$ $day^{-1} \cdot torr^{-1}$) varies with egg mass (W, in g) according to the equation (Ar & Rahn 1978):

$$GH_2O = 0.384\ W^{0.814} \qquad (6)$$

However, variation in GH_2O within a species or genus can be sizeable without a significant change in egg mass (Packard et al. 1977, Ar & Rahn 1978, Carey et al. 1983, Carey 1986, Carey et al. 1987). This phenomenon occurs because eggshell conductance to gases can be varied independently of egg mass. This variation most frequently is correlated with the gaseous conditions in the nest (see review by Carey 1983). The two major shell components of conductance to gases are shell thickness and functional pore

Table 1. Characteristics of eggs and shells of willow ptarmigan *Lagopus lagopus* collected at 720 m a.s.l. and white-tailed ptarmigan *Lagopus leucurus* collected between 3,600 and 4,100 m a.s.l. The P-values result from unpaired t-tests on the two means for each characteristic.

Characteristic	*Lagopus lagopus*			*Lagopus leucurus*			
	$\bar{x}$	SE	N	$\bar{x}$	SE	N	P
Egg conductance to water vapour (GH_2O, mg $\cdot$ $day^{-1} \cdot tor^{-1}$)	2.92	±0.168	(4)	2.50	±0.158	(5)	0.12
Shell permeability to water vapour (KH_20, cm^3 STP $\cdot$ $sec^{-1} \cdot cm^{-2} \cdot torr^{-1} \cdot 10^{-6}$)	1.024	±0.166	(4)	1.108	±0.014	(5)	0.45
Egg volume (cm^3)	21.26	±0.397	(4)	19.09	±0.404	(11)	0.01
Shell surface area (cm^2)	37.94	±0.471	(4)	35.295	±0.502	(11)	0.03
Shell thickness (mm)	0.193	±0.004	(4)	0.184	±0.004	(9)	0.23
Daily water loss in field (g $\cdot$ day^{-1})	0.159	±0.007	(4)	0.161	±0.013	(4)	0.87

area (a product of pore size and number (equation 1)). Since shell thickness does not vary significantly between species of ptarmigan, the functional pore area likewise must be similar. In every case studied thus far, changes in functional pore area in eggs of birds breeding over altitudinal gradients have been due to variation in the numbers of pores, not their sizes (Ar & Rahn 1985, Carey et al. 1987, Carey et al. 1989a,b)

The average barometric pressure at breeding locations of white-tailed ptarmigan (3,600-4,100 m) is about 60-65% of the sea level value (760 torr) while that of the willow ptarmigan is about 90% of the sea level value. Since the diffusion coefficient for water vapour varies inversely with barometric pressure (Paganelli et al. 1975), D should be about 25-30% greater for white-tailed ptarmigan eggs than for willow ptarmigan eggs. However, the average daily water losses from eggs incubated naturally by white-tailed and willow ptarmigan did not differ significantly ($P = 0.87$, see Table 1). Since the increased tendency of water vapour to diffuse at altitude has not been offset by a change in conductance, as observed in most other species breeding over altitudinal gradients (Carey et al. 1983, 1987, 1989a,b, 1991), the similarity in water loss may result from a lower average incubation temperature in white-tailed ptarmigan. If eggs cool more during foraging trips of white-tailed ptarmigan females than of willow ptarmigan hens or if white-tailed ptarmigan hens do not incubate as constantly in the early stages of incubation as willow ptarmigan hens, the increased diffusion coefficient for gases at altitude would be offset because temperature is a factor governing the rate of gas diffusion (equation 1). If the difference in incubation periods existing between naturally incubated willow (22 days) and white-tailed ptarmigan embryos (25 days) (Martin et al. 1993) disappears if white-tailed ptarmigan are incubated in an incubator at 37°C, differences in average incubation temperature should exist in the field.

The initial relative water content of the freshly laid egg and the final relative water content of the pipped embryo match within 1% (depending upon developmental mode) for all birds that have been investigated (Ar & Rahn 1980, Carey 1983). The relative initial water content and final pipped content of precocial eggs average about 75% (Ar & Rahn, 1980). While the importance of constancy of relative water content throughout incubation has not been resolved, Ar & Rahn (1980) concluded that maintenance of the relative water content is essential for embryonic development and survivability. Conceivably, birds breeding at high altitude could offset potentially higher rates of water loss at lower barometric pressure by laying eggs with a higher initial relative water content. However, no existing study on species breeding over altitudinal gradients has found any variation in fresh and final water contents (Carey et al. 1983, 1987, 1989a,b). If white-tailed ptarmigan lay eggs with an initial relative water content around 75%, the similarity in water loss between their eggs and those of willow ptarmigan indicates that embryos of both species develop in the same hydric environment, despite differences in the altitudes at which the eggs are laid. When the amount of water lost daily is multiplied by the average incubation period, and then divided by the fresh egg mass, the fractional water loss (the total amount of egg mass lost as water vapour during incubation to pipping) of willow ptarmigan is 15.6%. This value is well within the limits (11-23%) recorded for a variety of other birds breeding at low altitudes (Ar & Rahn 1980). While the fractional water loss cannot be calculated for white-tailed ptarmigan eggs without data on fresh egg mass, it is extremely unlikely that the fractional water loss of these eggs exceeds the 11-23% limits.

Although white-tailed and willow ptarmigan embryos develop in a similar hydric environment, white-tailed embryos experience restricted O_2 availability. Although the diffusion coefficient for O_2 increases with altitude, this effect only partially offsets the decrease in ambient partial pressure of O_2 (PO_2) at low P_B (Visschedijk et al. 1980). As a result, the PO_2 inside the air cell of the egg decreases as the altitude at which eggs are laid increases (Carey et al. 1982, 1989a, Carey, Dunin-Borkowski, Leon-Velarde, Espinosa & Monge 1994). Since the air cell PO_2 serves as the upper end of the gradient for diffusion of O_2 into the blood, and ultimately, into the cells, the decrease in air cell PO_2 causes a reduction in the rate of O_2 diffusion to the cells. Chicken *Gallus domesticus* embryos incubated at altitudes of up to 3,800 m exhibit decreased O_2 consumption, increased duration of incubation period, decreased hatchling mass, and increased embryonic mortality (Wangensteen, Rahn, Burton & Smith 1974). However, most wild birds (see Carey et al. 1989a for exception) breeding up to 4,100 m have physiological mechanisms for facilitating O_2 transport in hypoxic conditions, with the result that O_2 consumption, hatchling mass, incubation periods, and mortality rates are independent of altitude (Carey et al. 1982, 1994). These mechanisms include increased hemoglobin concentrations and hematocrits (proportion of red blood cells in blood volume) (Carey et al. 1993, 1994), but other mechanisms undoubtedly exist that remain to be described.

Our studies comparing white-tailed and willow ptarmigan embryos have investigated whether mechanisms exist that could facilitate O_2 transfer and utilization (Carey, Martin, May & Harper, unpubl. data). Oxygen consumption of white-tailed ptarmigan embryos measured at 4,200 m a.s.l. is similar to that of willow ptarmigan embryos at 720 m. These data indicate that white-tailed ptarmigan do have physiological mechanisms which facilitate O_2 transport and utilization in hypoxic conditions. Incubation periods of white-tailed ptarmigan average about three days longer than those of willow ptarmigan (Martin et al. 1993), but this effect may be due more to lower average incubation temperatures than to hypoxia. Hematocrits of white-tailed ptarmigan are significantly higher than those of willow ptarmigan embryos at all developmental stages except hatching (Dragon, Carey, Martin & Baumann, submitted). Heart and muscle citrate synthase activities (an enzyme which is an indicator of capacities of the tissue for aerobic production of ATP) of white-tailed ptarmigan embryos are significantly higher at comparable body masses than those of embryonic willow ptarmigan (Carey et al., unpubl. data). However, electrophoretic mobility of hemoglobin does not vary significantly among the two groups (Dragon et al., submitted).

When white-tailed ptarmigan eggs laid at 3,600-4,100 m were incubated for most of the incubation period at 1,600 m, hematocrit and citrate synthase activity in heart and muscle were indistinguishable from values for willow ptarmigan incubated at 720 m (Dragon et al., submitted, Carey et al., unpubl. data). Furthermore, the decrease in ATP and increase in 2,3-biphosphoglycerate in red blood cells of white-tailed ptarmigan embryos, a developmental change which fosters the change in O_2 affinity of hemoglobin necessary for O_2 supply to the growing embryo, occurs at a smaller embryonic mass in embryos incubated at altitudes higher than 1,600 m (Dragon et al., submitted). These data indicate that the higher hematocrits, greater activities of citrate synthase, and developmental timing of the appearance and disappearance of red blood cell organic phosphates of the white-tailed ptarmigan embryos at high altitudes are acclimatory responses to hypoxia, rather than genetically fixed characteristics. Our conclusions could be tested by measuring these parameters in white-tailed ptarmigan embryos laid in nests at much lower altitudes (1,200 m) in Alaska and British Columbia (Braun et al. 1993).

These acclimatory adjustments of white-tailed ptarmigan embryos, in addition to others that may exist, appear to be absolutely necessary for successful growth and development at high altitude. When white-tailed ptarmigan embryos incubated for most of the incubation period at 1,600 m were moved to 4,200 m, O_2 consumption of each embryo at 4,200 m was $^1/_3$-$^1/_2$ of the value for the same embryo tested earlier in the day at 1,600 m (Carey et al., unpubl. data).

Much more remains to be learned about how the design of avian eggshells and the physiological properties of avian embryos have been selected by multiple, but conflicting, requirements for gas exchange. The wide altitudinal distribution of ptarmigan make them a worthy subject for continuation of this research.

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