



*Research article*

## **Patterns of correlated character evolution in flightless birds: a phylogenetic approach**

JORGE CUBO<sup>1\*</sup> and WALLACE ARTHUR<sup>2</sup>

<sup>1</sup>*Equipe 'Formations squelettiques', UMR CNRS 8570, Université Paris 6, 2, Place Jussieu – Case 7077, F-75251 Paris Cedex 05, France;* <sup>2</sup>*Ecology Centre, School of Sciences, University of Sunderland, Sunderland, SR1 3SD, United Kingdom*  
(\*author for correspondence, tel.: +33-1-44-27-31-24; fax: +33-1-44-27-56-53;  
e-mail: cubo@ccr.jussieu.fr)

Received 13 February 2001; accepted 4 May 2001

Co-ordinating editor: P. Harvey

**Abstract.** Given a robust phylogeny for a particular higher taxon, it is possible to map the evolution of various character changes onto the phylogeny and study the extent to which they co-occur. Of particular interest are the questions of (a) whether particular morphological changes tend to accompany changes in ecology or behaviour to which they bear a functional relationship and (b) whether changes in those 'primary' morphological characters tend to be associated with correlated changes in other aspects of morphology, as would be expected given the high level of morphological integration that characterizes most organisms. Here we report a study of this kind, looking at morphological correlates of the evolution of flightlessness in birds, and using the concentrated changes test to determine whether associations are significant. We find that pectoral reduction, pelvic enlargement and changes in skull morphology significantly co-occur, and that these are usually achieved through heterochrony rather than other kinds of developmental reprogramming.

**Key words:** birds, concentrated changes test, developmental reprogramming, flightlessness, heterochrony, paedomorphosis, peramorphosis

### **Introduction**

Now that cladistic techniques and the addition of molecular to morphological data have resulted in robust phylogenies for at least a handful of higher taxa, it is possible to map the evolution of different characters onto these phylogenies. It is then possible to ask questions about the degree to which the evolution of different characters, or suites of characters, are correlated. When the characters concerned are a mixture of ecological and developmental ones, the endeavour to interconnect them falls at the fertile boundary between 'evo–devo' (evolutionary developmental biology; Hall, 1998) and what has been called 'historical ecology' (Brooks, 1985; Sheldon and Whittingham, 1997).

The degree to which ecological/behavioural changes and morphological/developmental ones go together can only be investigated when a particular kind of evolutionary change has recurred often within a higher taxon. For example, Wray and Bely (1994) conducted a study in which the developmental correlates of evolution from feeding to non-feeding echinoid larvae were examined. Such changes have occurred often (perhaps around 50 times) during the history of the echinoid clade (Emlet, 1990). Here, we provide a similar analysis of the developmental correlates of a flightless mode of life in birds, which has originated independently in at least 10 lineages (Cubo and Casinos, 1997).

### **Two questions**

It is important to be clear about the sort of questions that such an analysis seeks to answer. Specifically, we wish to distinguish between two sorts of questions:

1. Is a particular morphological change associated with a particular ecological change to a sufficient extent that we can be confident that the association is not simply a result of chance? (There is an established method for attempting to answer this question: the concentrated changes test (Maddison, 1990)).
2. Considering other morphological characters that we might expect to be correlated with the 'prime' character under consideration, is there evidence that these do indeed co-vary as expected? (This connects with the important issue of phenotypic integration, in which there is renewed interest (Wagner and Schwenk, 2000)).

The former question is the obvious starting point, though in a way the answer to it is less interesting, because often it is merely a confirmation of what is expected. So it comes as little surprise, for example, that an evolutionary switch in the echinoid clade from self-feeding to yolk-fed larvae is generally accompanied by (a) loss of larval feeding structures and (b) increased egg size (Wray and Bely, 1994). Equally, in relation to avian flightlessness, it is not surprising that a flightless mode of existence is generally associated with reduction in the size of the wing, not necessarily in absolute terms, but in relation to overall body size. Nevertheless, the independent origin of flightlessness in many avian clades (Cubo and Casinos, 1997; McCall et al., 1998) provides multiple evolutionary replicates which allow us to carry out a search for patterns of correlated character evolution, and so to address both the questions outlined above.

Raikow (1985) analyzed the incidence of flightlessness in birds and Livezey (1995) provided a survey of the occurrence in birds of both flightlessness – an ecological feature – and developmental patterns which are the outcome of heterochrony: paedomorphosis, the retention of ancestral juvenile characters in adult stages of descendants (Gould, 1977) and peramorphosis, the development of traits beyond that of the ancestral adult (McKinney and McNamara, 1991). The paedomorphic features of the pectoral apparatus of flightless birds are: small and unsculptured wing bones (James and Olson, 1983), obtuse scapulo-coracoid angles and disproportionate reduction of the distal wing elements relative to proximal elements (Livezey, 1995). On the other hand, stout hind limbs and massive pelvis are peramorphic features of the pelvic apparatus of flightless birds (James and Olson, 1983).

Although it is not surprising that, in Livezey's (1995) sample, a flightless mode of existence is always associated with a paedomorphic reduction of the pectoral apparatus, other associations between morphological changes and ecological changes are less evident, and the patterns of correlated character evolution need to be statistically tested. This is the case with the incidence of peramorphosis in the pelvic apparatus and the flightless condition in birds, which we will analyze below. This naturally leads to the second question posed above, and in relation to this we can ask in the case of flightless birds: do particular developmental changes in other parts of the body tend to co-occur with paedomorphic reduction of pectoral development or with over-development of pelvic apparatus? In the analysis below, we focus on skull morphology, a structure for which there seems at first sight little reason to expect a specific connection with flightlessness.

### **The analysis**

One version of our first question is as follows: Is peramorphosis affecting the pelvic apparatus associated with the flightless condition to a sufficient extent that we can be confident that the 'association' is not simply a result of chance? To answer this question, we should perform a comparative analysis framed in a phylogenetic context (Harvey and Pagel, 1991). For this, we will use the concentrated changes test, which allows 'to test for correlated evolution of two characters by asking whether changes in the first character are significantly concentrated on those branches on which the second character has a specified state' (Maddison, 1990).

Peramorphosis in the pelvic apparatus was diagnosed by Livezey (1995) through analysis of the features produced by changes in developmental timing and rates and not by analysis of changes in developmental timing and rates themselves (see Gould, 2000, for a review of this method of heterochronic

detection). The flightless taxa which have been analyzed are those reviewed by Livezey (1995, Table 9.2), excluding those species from which only subfossil remains are available. Three phylogenetic hypotheses of major groups of birds are currently available: (a) a phylogeny based on morphological data (Cracraft, 1988), (b) a phylogeny based on DNA–DNA hybridization (Sibley and Ahlquist, 1990) and (c) a phylogeny based on mitochondrial DNA sequences (Mindell *et al.*, 1997). Cracraft's (1988) phylogeny has been widely accepted, but it has several unresolved polytomies and it does not consider Psittaciformes, an order of interest in our study. Sibley and Ahlquist's (1990) phylogeny is controversial; however, it has been shown that it is generally valid when conducting large-scale analyses (Moore and Cotgreave, 1994). Finally, the phylogeny of Mindell *et al.* (1997) does not consider a number of orders of interest in this analysis, namely Psittaciformes, Columbiformes and Podicipediformes. We have used Cracraft's (1988) phylogenetic relationships of major groups of birds to carry out our analysis, with the only exception of the phylogenetic position of Psittaciformes, which has been taken from Sibley and Ahlquist (1990). Cracraft's (1988) tree has been constructed with morphological characters other than those of interest in this study and, therefore, there is not a problem of circularity. We used closely related flighted congeners or confamilials to estimate the ancestral flighted condition of each flightless species. In the case of ratites (Palaeognathiformes), it was assumed that the ancestral condition has been retained by two species of tinamous, which are the flighted sister-group of ratites.

To perform the concentrated changes test (Maddison, 1990), the incidence of flightlessness has been traced onto the phylogenetic tree (Fig. 1(a), black branches). The flightless condition has arisen 10 independent times in our tree. Afterwards, the occurrence of peramorphic features of the pelvic apparatus (Livezey, 1995) has also been traced onto the tree (Fig. 1(a), horizontal bars). The concentrated changes test (Maddison, 1990) gives us the probability, under the null hypothesis that gains and losses are randomly distributed over the branches, that seven gains of 'peramorphic pelvic apparatus' would occur on flightless lineages, given seven gains of 'peramorphic pelvic apparatus' occur in the whole clade. This probability is  $p = 0.001$  and, therefore, we should conclude that the association between the flightless condition and peramorphosis in pelvic apparatus is not the result of chance.

Interestingly, many flightless birds show peramorphosis in skull morphology, an association which, as noted above, there seems little reason to expect. This connects with question 2: do particular developmental changes in other parts of the body of flightless birds tend to co-occur with pedomorphic reduction of pectoral development or with over-development of pelvic apparatus? Heterochrony often leads to a whole array of derived, but otherwise unrelated, character states. In this context, the following question arose: Is peramorphosis in

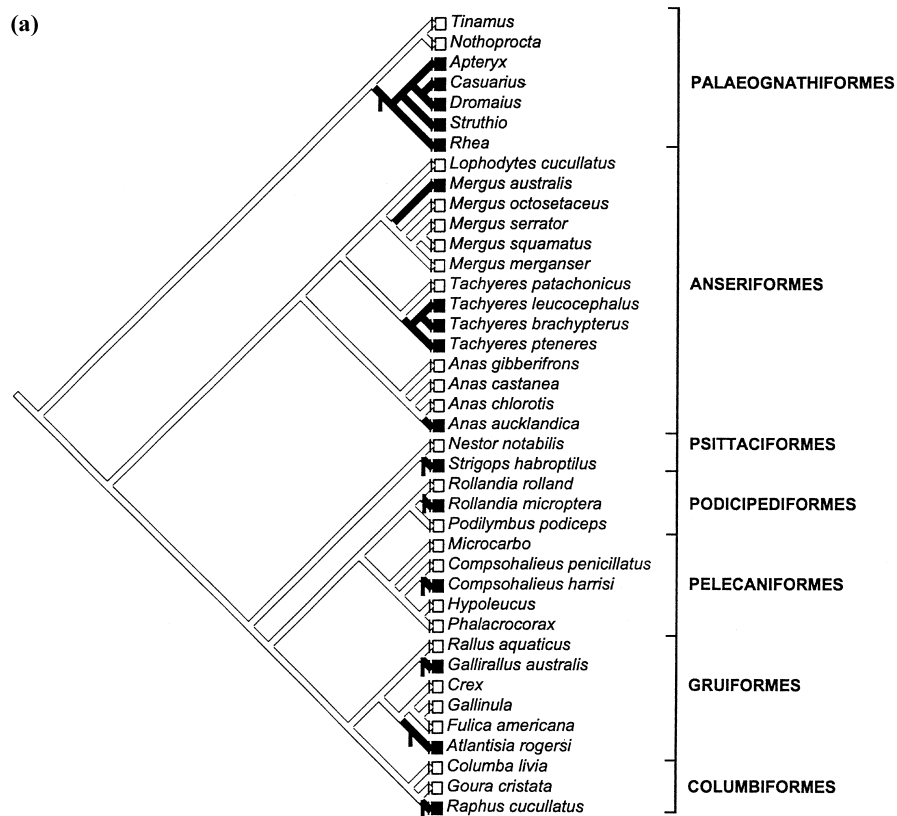


Figure 1. Optimization, using parsimony, of ecological and morphological characters onto a phylogeny of birds. Phylogenetic relationships among major groups of birds are those proposed by Cracraft (1988) with the only exception of the phylogenetic position of Psittaciformes, which has been taken from Sibley and Ahlquist (1990). Phylogenetic relationships within orders are modified from: for Palaeognathiformes, Lee *et al.* (1997); for Anseriformes, Livezey and Humphrey (1986), Livezey (1989, 1990) and McCracken *et al.* (1999); for Columbiformes, Janoo (1997); for Gruiformes, Livezey (1998); and for Phalacrocoracidae (Pelecaniformes), Siegel-Causey (1988). Morphological data are those reviewed by Livezey (1995, Table 9.2), excluding species from which only subfossil remains are available. (a) An optimization of the incidence of flightlessness or incipient flightless condition (black branches) and the incidence of peramorphosis affecting the pelvic apparatus (horizontal bars). (b) An optimization of the presence of peramorphosis affecting the pelvic apparatus (black branches) and the presence of peramorphosis affecting the skull (horizontal bars).

skull structure linked by correlated development with peramorphosis in the pelvic apparatus? To answer this question, the independent variable (the incidence of peramorphosis in the pelvic apparatus (Livezey, 1995)) should be traced onto the phylogenetic tree (Fig. 1(b), black branches). The presence of peramorphic features in the skull (Livezey, 1995) has also been traced onto the tree (Fig. 1(b), horizontal bars). The concentrated changes test (Maddison, 1990) gives us the probability that four gains of 'peramorphic skull' would occur

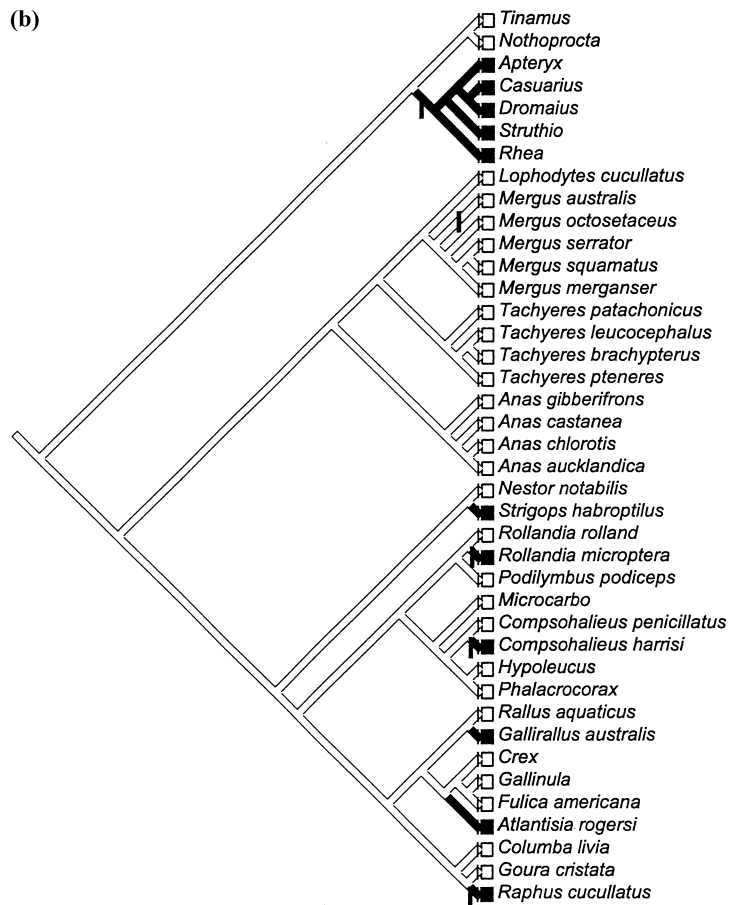


Figure 1. Continued.

on lineages which show peramorphosis in the pelvic apparatus, given five gains of ‘peramorphic skull’ occur in the whole clade, and given the null model that changes are randomly distributed among the branches of the clade. This probability is  $p = 0.003$  and, therefore, we should conclude that the association between peramorphosis in pelvic apparatus and peramorphosis in skull is not the result of chance. Rather, peramorphosis in skull structure would appear to be linked by correlated development with peramorphosis in the pelvic apparatus.

## Discussion

As stated by Sheldon and Whittingham (1997, p. 287), ‘Given the difficulty of determining past environmental conditions and organismal interactions, most

inferences of adaptation quite reasonably rely on correlations of character changes in a phylogenetic context'. In this framework an adaptation has been defined as an apomorphic feature that evolved in response to an apomorphic function (Coddington, 1994).

What would be the ultimate causation of the association between flightless mode of existence and the paedomorphic reduction of the pectoral apparatus? It has been hypothesized that '... if there is not continued selection to warrant the energy expenditure in the embryogenesis of these complex structures, they will tend to be lost, whether they are muscles, bones, or feathers. Many of these adaptations, then, help the flightless bird save energy, both during embryogenesis and in adult life, by removing the burden of unwanted structures that hinder locomotion and by reducing the metabolic energy spent maintaining them' (Feduccia, 1996, p. 258). The fact that a flightless mode of existence is always associated with a paedomorphic reduction of the pectoral apparatus suggests that paedomorphosis of pectoral apparatus (evo–devo pattern) is an adaptation (evolutionary process) for a more efficient use of energy both during embryogenesis and in adult life, which has been repeatedly selected in the ecological contexts which allow the acquisition of the flightless condition. Regarding the proximal causation of reduction in the relative size of the wing, the development of forelimbs is delayed relative to the development of hindlimbs in birds (Cane, 1993; Castanet *et al.*, 1996; Castanet *et al.*, 2000), in such a way that the truncation (progenesis) or retardation (neoteny) of general development is likely to produce reduction of the size of the pectoral apparatus.

On the other hand, it has been shown in flightless birds that 'peramorphosis of the pelvic limb is especially conspicuous in some groups, associated with a 'compensatory' shift in appendicular specialization' (Livezey, 1995, p. 184). Does the flightless condition promote a compensatory shift in appendicular growth? If this is the case, we expect to find an association between flightless condition and over-development (peramorphosis) of pelvic apparatus. As noted above, the concentrated changes test (Maddison, 1990) shows that the association between this ecological change and this trait is significant. Therefore, we can conclude that cases of peramorphosis of pelvic apparatus (evo–devo pattern) are adaptations (evolutionary process) associated with a compensatory shift in appendicular specialization of hind limbs to running or to foot-propelled diving, which have been repeatedly selected in many clades of flightless birds. We hope we have shown that the optimization of evo–devo patterns and ecological characters onto phylogenetic trees generated independently can allow the identification of the evolutionary processes which generate these patterns.

Finally, given two or more developmental ways of producing a certain morphology, does one of them tend to occur more often than the other? In our case

study, heterochrony underlies both reduction of pectoral apparatus and overdevelopment of pelvic apparatus of flightless birds. However, it has recently been shown that non-heterochronic developmental changes can underlie morphological heterochrony (Cubo, 2000; Cubo *et al.*, 2000). For instance, an evolutionary change in the number of proliferating cells at growth plates, which fits into the category of ‘heteroposity’ (Regier and Vlahos, 1988; Raff and Wray, 1989) or ‘heterometry’ (Arthur, 2000), underlies morphological acceleration (peramorphosis) in the tarsometatarsus of the Ardeidae (Cubo *et al.*, 2000). Both heterochrony and heteroposity/heterometry are subcategories within the overall phenomenon of ‘developmental reprogramming’ (Arthur, 2000; an expanded view of the ‘ontogenetic repatterning’ of Roth and Wake, 1985) – that is, evolutionary changes in developmental parameters. Future research on the developmental basis of the reduction of the pectoral apparatus and the overdevelopment of the pelvic apparatus should allow elucidation of whether heterochrony is the only type of developmental reprogramming involved in the evolution of flightless birds. Also, if other types of reprogramming can occur, such studies may allow the elucidation of whether one type of reprogramming tends to occur more often than the other types, which relates to the question of whether biases in development help to drive evolution in certain directions (Raff, 2000; W. Arthur, submitted for publication).

### Acknowledgements

We thank Michel Laurin (UMR CNRS 8570, Paris, France) for allowing us the use of MacClade 4.0 and for valuable discussions on the methods used in this manuscript.

### References

- Arthur, W. (2000) The concept of developmental reprogramming and the quest for an inclusive theory of evolutionary mechanisms. *Evol. Dev.* **2**, 49–57.
- Brooks, D.R. (1985) Historical ecology: a new approach to studying the evolution of ecological associations. *Ann. Missouri Bot. Garden* **72**, 660–680.
- Cane, W.P. (1993) The ontogeny of posterianal integration in the common tern, *Sterna hirundo*. *Evolution* **47**, 1138–1151.
- Castanet, J., Curry Rogers, K., Cubo, J. and Boisard, J.J. (2000) Periosteal bone growth rates in extant ratites (ostriche and emu). Implications for assessing growth in dinosaurs. *C.R. Acad. Sci. Paris, Sci. Vie* **323**, 543–550.
- Castanet, J., Grandin, A., Abourachid, A. and de Ricqlès, A. (1996) Expression de la dynamique de croissance dans la structure de l’os périostique chez *Anas platyrhynchos*. *C.R. Acad. Sci. Paris, Sci. Vie* **319**, 301–308.
- Coddington, J.A. (1994) The roles of homology and convergence in studies of adaptation. In P. Eggleton and R.I. Vane-Wright (eds) *Phylogenetics and Ecology*. Academic Press, London.

- Cracraft, J. (1988) The major clades of birds. In M.J. Benton (ed.) *The Phylogeny and Classification of the Tetrapods, Volume 1: Amphibians, Reptiles, Birds*. Clarendon Press, Oxford.
- Cubo, J. (2000) Process heterochronies in endochondral ossification. *J. Theor. Biol.* **205**, 343–353.
- Cubo, J. and Casinos, A. (1997) Flightlessness and long bone allometry in Palaeognathiformes and Sphenisciformes. *Neth. J. Zool.* **47**, 209–226.
- Cubo, J., Fouces, V., Gonzalez-Martin, M., Pedrocci, V. and Ruiz, X. (2000) Nonheterochronic developmental changes underlie morphological heterochrony in the evolution of the Ardeidae. *J. Evol. Biol.* **13**, 269–276.
- Emler, R.B. (1990) World patterns of developmental mode in echinoid echinoderms. In *Advances in Invertebrate Reproduction*. M. Hoshi and O. Yamashita (eds), Elsevier, Amsterdam, pp. 329–335.
- Feduccia, A. (1996) *The Origin and Evolution of Birds*. Yale University Press, New Haven.
- Gould, S.J. (1977) *Ontogeny and Phylogeny*. The Belknap Press of Harvard University Press, Cambridge, Massachusetts.
- Gould, S.J. (2000) Of coiled oysters and big brains: how to rescue the terminology of heterochrony, now gone astray. *Evol. Dev.* **2**, 241–248.
- Hall, B.K. (1998) *Evolutionary Developmental Biology*. Chapman & Hall, London.
- Harvey, P.H. and Pagel, M.D. (1991) *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.
- James, H.F. and Olson, S.L. (1983) Flightless birds. *Nat. Hist.* **92**, 30–40.
- Janoo, A. (1997) La position phylogénétique du Dodo (*Raphus cucullatus* L.) et du Solitaire (*Pezophaps solitaria* Gm.): l'évolution du complexe coracoïdien au sein des Ornithurae, centrée sur les Neognathae (Division 4 et 5 *sensu* Cracraft, 1981). PhD Thesis, Muséum National d'Histoire Naturelle, Paris.
- Lee, K., Feinstein, J. and Cracraft, J. (1997) The phylogeny of ratite birds: resolving conflicts between molecular and morphological data sets. In D.P. Mindell (ed.) *Avian Molecular Evolution and Systematics*. Academic Press, San Diego.
- Livezey, B.C. (1989) Phylogenetic relationships and incipient flightlessness of the extinct Auckland Islands merganser. *Wilson Bull.* **101**, 410–435.
- Livezey, B.C. (1990) Evolutionary morphology of flightlessness in the auckland islands teal. *Condor* **92**, 639–673.
- Livezey, B.C. (1995) Heterochrony and the evolution of avian flightlessness. In K.J. McNamara (ed.) *Evolutionary change and heterochrony*. John Wiley & Sons Ltd, Chichester.
- Livezey, B.C. (1998) A phylogenetic analysis of the Gruiformes (Aves) based on morphological characters, with an emphasis on the rails (Rallidae). *Phil. Trans. R. Soc. Lond. B* **353**, 2077–2151.
- Livezey, B.C. and Humphrey, P.S. (1986) Flightlessness in steamer-ducks (Anatidae: *Tachyeres*): its morphological bases and probable evolution. *Evolution* **40**, 540–558.
- Maddison, W.P. (1990) A method for testing the correlated evolution of two binary characters: are gains or losses concentrated on certain branches of a phylogenetic tree? *Evolution* **44**, 539–557.
- McCall, R.A., Nee, S. and Harvey, P.H. (1998) The role of wing length in the evolution of avian flightlessness. *Evol. Ecol.* **12**, 569–580.
- McCracken, K.G., Harshman, J., McClellan, D.A. and Afton, A.D. (1999) Data set incongruence and correlated character evolution: an example of functional convergence in the hind-limbs of stiff-tail diving ducks. *Syst. Biol.* **48**, 683–714.
- McKinney, M.L. and McNamara, K.J. (1991) *Heterochrony: the evolution of ontogeny*. Plenum, New York.
- Mindell, D.P., Sorenson, M.D., Huddleston, C.J., Miranda, H.C., Knight, A., Sawchuk, S.J. and Yuri, T. (1997) Phylogenetic relationships among and within select avian orders based on mitochondrial DNA. In D.P. Mindell (ed.) *Avian Molecular Evolution and Systematics*. Academic Press, San Diego.
- Mooers, A. and Cotgreave, P. (1994) Sibley and Ahlquist's tapestry dusted off. *Trends Ecol. Evol.* **9**, 458–459.
- Raff, R.A. (2000) Evo-devo: the evolution of a new discipline. *Nature Reviews Genetics* **1**, 74–79.
- Raff, R.A. and Wray, G.A. (1989) Heterochrony: developmental mechanisms and evolutionary results. *J. Evol. Biol.* **2**, 409–434.

- Raikow, R.J. (1985) Locomotor system. In A.S. King and J. McLellund (eds.) *Form and Function in Birds, Vol. 3*. Academic Press, London.
- Regier, J.C. and Vlahos, N.S. (1988) Heterochrony and the introduction of novel modes of morphogenesis during the evolution of moth choriogenesis. *J. Mol. Evol.* **28**, 19–31.
- Roth, G. and Wake, D.B. (1985) Trends in the functional morphology and sensorimotor control of feeding behavior in salamanders: an example of the role of internal dynamics in evolution. *Acta Biotheoretica* **34**, 175–192.
- Sheldon, F.H. and Whittingham, L.A. (1997) Phylogeny in studies of bird ecology, behavior and morphology. In D.P. Mindell (ed.) *Avian Molecular Evolution and Systematics*. Academic Press, San Diego.
- Sibley, C.G. and Ahlquist, J.E. (1990) *Phylogeny and classification of birds*. Yale University Press, New Haven, Connecticut.
- Siegel-Causey, D. (1988) Phylogeny of the Phalacrocoracidae. *Condor* **90**, 885–905.
- Wagner, G.P. and Schwenk, K. (2000) Evolutionarily stable configurations: functional integration and the evolution of phenotypic stability. In M.K. Hecht *et al.* (eds) *Evolutionary Biology* 31. Academic/Plenum, New York.
- Wray, G.A. and Bely, A.E. (1994) The evolution of echinoderm development is driven by several distinct factors. *Development* (Suppl) 97–106.