

sa being "less dark" than on other islands. Female *G. difficilis* also have rufous wing bars, absent in *G. fuliginosa*, but the trait occurs in very low frequency in the Genovesa and Pinta populations. The under-tail coverts in adult male *G. difficilis* are often rufous-tipped, whereas the coverts are white-tipped in *G. fuliginosa*. Lack noted considerable variation among *G. difficilis* populations in the frequency of the rufous tip: in 100% of the Darwin and Wolf specimens, 50% of those from San Salvador and Santa Cruz, and 10% of those from Pinta and Genovesa.

The small finch on Isla Genovesa, Galápagos, is similar to *G. fuliginosa* in overall body size, but in shape it is very much a *G. difficilis*. Song and plumage variables are consistent with this result. We conclude that the data best fit Lack's (1947) classification, and that the taxonomic revision proposed by Vagvolgyi and Vagvolgyi (1989) is unsupported.

LITERATURE CITED

- ABBOTT, I., L. K. ABBOTT, & P. R. GRANT. 1977. Comparative ecology of Galápagos ground finches (*Geospiza* Gould): evaluation of the importance of floristic diversity and interspecific competition. *Ecol. Monogr.* 47: 151-184.
- BOAG, P. T. 1983. The heritability of external morphology in the Darwin's finches (Geospizinae) of Daphne Major Island, Galápagos. *Evolution* 37: 877-894.
- BOWMAN, R. I. 1983. The evolution of song in Darwin's finches. Pp. 237-537 in *Patterns of evolution in Galápagos organisms* (R. I. Bowman, M. Berson, and A. E. Leviton, Eds.). San Francisco, Pacific Div. Am. Assoc. Advmt. Sci.
- GRANT, P. R. 1983a. Inheritance of size and shape in a population of Darwin's finches, *Geospiza conirostris*. *Proc. R. Soc. London, B* 220: 219-236.
- . 1983b. The role of interspecific competition in the adaptive radiation of Darwin's finches. Pp. 187-199 in *Patterns of evolution in Galápagos organisms* (R. I. Bowman, M. Berson, and A. E. Leviton, Eds.). San Francisco, Pacific Div. Am. Assoc. Advmt. Sci.
- . 1986. Ecology and evolution of Darwin's finches. Princeton, Princeton Univ. Press.
- , I. ABBOTT, D. SCHLUTER, R. L. CURRY, & L. K. ABBOTT. 1985. Variation in the size and shape of Darwin's finches. *Biol. J. Linn. Soc.* 25: 1-39.
- LACK, D. 1947. Darwin's finches. Cambridge, Cambridge Univ. Press.
- LANDE, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. *Evolution* 33: 402-416.
- LYNCH, J. 1989. Phylogenetic hypotheses under the assumption of neutral quantitative-genetic variation. *Evolution* 43: 1-17.
- PIMENTEL, R. A. 1979. Morphometrics, the multivariate analysis of biological data. Dubuque, Kendall/Hunt.
- RATCLIFFE, L. M. 1981. Species recognition in Darwin's ground finches (*Geospiza*, Gould). Ph.D dissertation, McGill University, Montreal, Canada.
- SCHLUTER, D. 1984. Morphological and phylogenetic relations among the Darwin's finches. *Evolution* 38: 921-930.
- , & P. R. GRANT. 1982. The distribution of *Geospiza difficilis* in relation to *G. fuliginosa* in the Galápagos Islands: tests of three hypotheses. *Evolution* 36: 1213-1226.
- , & ———. 1984. Ecological correlates of morphological evolution in a Darwin's finch, *Geospiza difficilis*. *Evolution* 38: 856-869.
- VAGVOLGYI, J., & M. W. VAGVOLGYI. 1989. The taxonomic status of the Small Ground-Finch, *Geospiza* (Aves: Emberizidae) of Genovesa Island, Galápagos, and its relevance to interspecific competition. *Auk* 106: 144-148.
- YANG, S., & R. PATTON. 1981. Genic variability and differentiation in Galápagos finches. *Auk* 98: 230-242.

Received 3 November 1989, accepted 27 November 1989.

Response to Schluter, Ratcliffe, and Grant

JOSEPH VAGVOLGYI¹ AND MARIA W. VAGVOLGYI¹

Schluter, Ratcliffe, and Grant (1991) argue that the Small Genovesa Ground-Finch should be classified as *Geospiza difficilis* because the species are similar in shape, and—more so than size—shape is an independent,

reliable, and important indicator of taxonomic and evolutionary relationships.

The classification of Darwin's finches is based on size as well as shape characters. Lack (1947: 81, 88) as well as Grant and Grant (1989: 377) emphasized this: "Beak size and shape . . . have been identified as important in the evolutionary diversification [of Darwin's Finches] . . . since species differ from each other

¹ Biology Department, B-204, College of Staten Island, City University of New York, 715 Ocean Terrace, Staten Island, New York 10301 USA.

in beak dimensions, whereas other traits, such as plumage and behavioral characteristics associated with reproduction, are similar or identical." Thus one would expect that the value of size characters be recognized by Schluter, Ratcliffe, and Grant (1991).

Special importance is attached by Schluter et al. to the trait beak length from the tip to the point where beak depth is 4 mm (L@4). However, this character is a simple, linear measurement, with continuous variation and distribution around a mean, similar to Schluter et al.'s (1991) size characters. Furthermore, they disregard that the shape of the beak can be described by size characters, beak length or culmen and beak depth. Large culmen and small depth values indicate pointed beak shape; the reverse values, blunt. Many authors, including Grant and Grant (1989), utilized this method. The relationship between beak length at 4 mm, on the one hand, and culmen and beak depth on the other, is obvious. The separation of size and shape is forced. This also means that our classification of the Small Genovesa Ground-Finch as *G. fuliginosa* is based on size as well as shape characters.

Single characters may conceivably be used to separate two species. Beak length to the point where beak depth is 4 mm does not, however, appear to be adequate for this purpose. In Schluter et al.'s (1991) figure 1, the means of three populations of *G. fuliginosa* fall close to *G. difficilis*, which indicates potentially wide overlaps. Schluter et al. do not provide information on the variation of this character to judge the extent of overlap.

From their presumption that shape evolved slower than size, combined with their notions on niche shift and competition, the evolutionary scenario on Genovesa Island emerges as follows. *Geospiza difficilis* colonized Genovesa Island, ecologically suited for its competitor, *G. fuliginosa*. The latter species was absent on this island, which allowed *G. difficilis* to shift to its rival's habitat and utilize its food. Consequently it became small like *G. fuliginosa*, but it retained a *difficilis*-type pointed beak, and thus it gave rise to the extant population. One problem with this scenario is that the envisioned shift from the mainly insectivorous diet of *G. difficilis* to the primarily granivorous diet of *G. fuliginosa* should have speeded the acquisition of the *fuliginosa*-type blunt beak, rather than conserving the *difficilis*-type pointed beak. Conservatism of beak shape should be anathema to supporters of character displacement in *Geospiza*, among them Schluter et al., who believe that the beak promptly and precisely tracks the changes occurring in the food supplies.

Another problem is the time course. Grant and Grant (1989) reported significant changes in the beak shape in *G. conirostris* on Genovesa Island over 3 yr, due to extreme climatic fluctuations that caused reduction in the fruits and seeds of *Opuntia*. Specimens with relatively long culmen "... as predicted, were at a se-

lective disadvantage. Only beak length was a target of selection . . ." (Grant and Grant 1989: 392; italics ours). If so, it is reasonable to assume that the Small Genovesa Ground-Finch was subject to comparable climatic and ecological fluctuations, hence also to rapid morphological changes. Furthermore, the vegetation on San Cristóbal Island, and presumably on the entire archipelago, has been stable for more than 9,000 yr (Colinvaux and Schofield 1976). Species divergence times in *Geospiza* have been estimated (Yang and Patton 1981) in excess of 50,000 yr, and *G. difficilis* is considered by Schluter et al. an old species. If so, the potential age of the Genovesa population may exceed by several orders of magnitude the time requirements for significant morphological change. On these grounds, we suggest that evidence is lacking to support Schluter et al.'s (1991) assumption that beak shape on Genovesa Island behaved more conservatively, or that it could serve as a more reliable evolutionary indicator than the other characters.

Another important issue is the inconsistency between Grant's earlier data and Schluter et al.'s main result, that beak shape clearly aligns the Genovesa finches with *G. difficilis*. Grant used the Small Ground-Finches on Genovesa and Española islands to support competitive displacement and competitive release. He stated (1986: 295) that "The evidence for this is morphological. *G. fuliginosa* on Española and *G. difficilis* on Genovesa have similar beaks." And, "On Genovesa, where . . . the small seed-eater (*G. fuliginosa*) . . . [is] absent, *G. difficilis* has evolved a very *fuliginosa*-like bill and body size." Moreover, based on developmental data, he stated (1986: 112) that "*G. fuliginosa* on Marchena and *G. difficilis* on Genovesa have identical relative growth trajectories and stop at identical points; hence adults of the two species have the same bill proportions . . . *G. difficilis*, on Wolf, grows along the same relative growth trajectory [as on Genovesa] but between different starting and stopping points, with the results that adults in the two populations have different bill proportions."

Schluter et al. (1991) made no attempt to reconcile these measurements, proportions, growth trajectories, and the resulting conclusions with their current assessment. Furthermore, the notions of character displacement and character release on Genovesa and Española islands are based on a Genovesa population that looks like *G. fuliginosa* but is *G. difficilis*. We argued against this view on the ground that the Genovesa population is a *bona fide* representative of *G. fuliginosa*. Schluter et al. aimed at countering this argument with their current assessment. In the process, they ignored the evidence they had used earlier to support their hypothesis on competitive displacement and release.

Our conclusion (Vagvolgyi and Vagvolgyi 1989) that the Genovesa finches possess the morphology of *G. fuliginosa* because they have evolved from *G. fuliginosa* ancestors is based on solid morphological grounds, and it is supported by the lack of any mor-

phological convergence on *G. fuliginosa* by the Darwin and Wolf populations. We believe that Schluter et al.'s arguments against it are implausible.

LITERATURE CITED

- COLINVAUX, P. A., & E. K. SCHOFIELD. 1976. Historical ecology in the Galápagos Islands. I. A Holocene pollen report from El Junco lake, Isla San Cristóbal. *J. Ecol.* 64: 989-1012.
- GRANT, B. R., & P. R. GRANT. 1989. Natural selection in a population of Darwin's finches. *Am. Nat.* 133: 377-393.
- GRANT, P. R. 1986. Ecology and evolution of Darwin's finches. Princeton, Princeton Univ. Press.
- LACK, D. 1947. Darwin's finches. Cambridge, Cambridge Univ. Press.
- SCHLUTER, D., L. M. RATCLIFFE, & P. R. GRANT. 1991. The taxonomic status of the Small Genovesa Ground-Finch in the Galápagos. *Auk* 108: 201-204.
- VAGVOLGYI, J., & M. W. VAGVOLGYI. 1989. The taxonomic status of the Small Ground-Finch, *Geospiza* (Aves: Emberizidae) of Genovesa Island, Galápagos, and its relevance to interspecific competition. *Auk* 106: 144-148.
- YANG, S. Y., & J. L. PATTON. 1981. Genic variability and differentiation in Galápagos finches. *Auk* 98: 230-242.

Received 3 August 1990, accepted 8 August 1990.

The Use of Flow Cytometry for Rapid Identification of Sex in Birds

TERRENCE R. TIERSCH,¹ RONALD L. MUMME,^{1,2} ROBERT W. CHANDLER,³ AND DEAN NAKAMURA⁴

Unambiguous identification of the sex of live birds is critical in numerous areas of avian research, including studies of alternative reproductive tactics, sex-ratio manipulation, and conservation biology (e.g. van Rhijn 1973, Snyder and Snyder 1989, Stamps 1990). However, identification of sex can be problematic for researchers dealing with young birds or sexually monomorphic species. Techniques currently available for identification of sex, primarily based on cytogenetics or biochemical genetics, are time-consuming, expensive, or require considerable amounts of tissue. The use of sex-specific DNA probes can overcome some of these shortcomings (Quinn et al. 1990), but this technique is time-consuming also, and probes may not be equally effective with DNA of species from divergent taxonomic groups. Other techniques such as laparotomy do not always work with nestlings or sexually immature birds, are potentially stressful, and may be inadvisable when dealing with threatened or endangered species.

Flow cytometry has been used to measure nuclear DNA content in a wide variety of organisms (e.g. Tiersch et al. 1989). In addition, flow cytometry has been used to identify differences in the DNA content of male and female humans (Deaven 1982, Elias et al. 1988) and other mammals (Kent et al. 1988). Recently, Nakamura et al. (1990) have developed a rapid and inexpensive procedure for sexing live birds through the use of flow cytometry. This procedure allows sex to be assigned on the basis of small but consistently measurable differences in the nuclear DNA content of males and females. Nuclear DNA content is a sexually dimorphic trait in birds because (1) in those species with heteromorphic sex chromosomes, the W chromosome is consistently smaller than the Z chromosome, and (2) males are homogametic (ZZ) and females are heterogametic (ZW). Our purpose in this commentary is to introduce flow cytometry to research ornithologists who might find the technique useful for the identification of sex in live birds.

The flow cytometer measures fluorescence, size, and granularity of cells. Most uses to date have been in areas of medicine (see review by Lovett et al. 1984). Flow cytometry has been applied to the study of cell surface receptors, cell pH, DNA synthesis, characteristics of the cell membrane, DNA base ratios, various cell and nuclear proteins and ions, phagocytosis and oxidative burst, cell RNA content, chromatin structure, and cytoskeletal organization. Other applications include karyotyping, testing for the effects of environmental mutagens (e.g. Deaven 1982, Bickham et al. 1988) and the detection of abnormalities in ploidy level (e.g. Allen 1983). The flow cytometer, also

¹ Ecological Research Center, Department of Biology, Memphis State University, Memphis, Tennessee 38152 USA.

² Present address: Department of Biology, Allegheny College, Meadville, Pennsylvania 16335 USA.

³ Puckett Laboratory, 4200 Mamie Street, Hattiesburg, Mississippi 39402 USA.

⁴ The National Law Center, George Washington University; Sughrue, Mion, Zinn, Macpeak & Seas, 2100 Pennsylvania Avenue NW, Suite 800, Washington, D.C. 20037 USA.