not significantly different) between these females (table 2). Thus there were no apparent residual effects of the space experience in these later generations.

These data suggest that there were some immediate affects of weightlessness on female houseflies emerging in space. These effects seem to be limited to the rate of egg development and did not affect either the number of eggs per each egg batch or the survival of offspring from these eggs. These differences were not owing to differences in size of female flies. Also, these flies were not provided an appropriate protein diet during the flight necessary for egg development[9] and thus egg development occurred after the flight at normal gravity conditions. Hence, there was a presumed direct affect of weightlessness on ovarian development that altered the rate of subsequent oviposition. If these effects are indeed due to the shuttle experience per se, they represent the first documentation of reduced reproductive ability in a complex organism exposed to space travel. These limited data at least suggest that additional investigation is warranted.

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2 Current address: Department of Zoology, Miami University-Hamilton, 1601 Peck Blvd, Hamilton, Ohio 45011, USA.
3 Rau, K.G., and Kalthoff, K., Nature *287* (1983) 635.
4 Miquel, J., and Philpott, D.E., Physiologist, Wash. *21* (1978) 80.
5 Leppla, N.C., Nelson, T.E., Peterson, J.R., and Adams, G.W., Bull. ent. Soc. Am. *29* (1983) 10.
6 Bryant, E.H., Ecology *50* (1969) 1049.
7 Bryant, E.H., Evolution *31* (1977) 580.
8 Siegel, S., Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York 1956.
9 Chapman, R.F., The Insects Structure and Function, 3rd edn. Harvard Univ. Press, Cambridge, Mass., 1982.

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Immunological systematics of the extinct quagga (Equidae)

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Summary. It has been debated whether the extinct quagga was a distinct fourth species of African zebra or whether it was merely the southern variant of the Plains zebra *(Equus burchelli)*. Using a radioimmunoassay (RIA) technique, we have shown that proteins remaining in quagga skins from museums are much more similar to serum proteins of the Plains zebra than to those of the other two extant zebras.
Key words. Quagga; zebra; horses, equids, radioimmunoassay; molecular evolution; proteins.

The quagga *(Equus quagga quagga)* was once the most numerous zebra-like animal found in southern Africa. Like the plains bison of North America, this large mammal was found in herds of inestimable size, but human activities destroyed their habitat, and human predation for skins and meat decimated their numbers[1]. The last quagga died alone in her stall at the Amsterdam zoo on 12 August 1883[2]. Only 23 quagga skins are now known to exist in museums[3].

Some controversy surrounds quagga systematics. The dominant hypotheses suggest that it represented either the fourth species of African zebra or, alternatively, that the clinal variation in striping pattern of the Plains zebra, *Equus burchelli,* included as its southernmost variant the quagga[4-8]. Using radioimmunoassay (RIA), we have shown that immunologically quagga skin is very similar to skin and serum of the Plains zebra.

Previous systematic analyses of zebras have depended largely on ranges and striping patterns. The quagga existed in Cape Province, south of the Orange River, at the southern extension of the range of Plains zebras, which in turn were subdivided by various systematists into between six and more than 30 races. The quagga was first described by Gmelin in 1788 as a distinct species[9]. The sportsman W.C. Harris also considered it as a distinct species whose range overlapped that of the Plains zebra in the Orange Free State[4]. Our present confusion is compounded by the fact that the terms quagga and zebra were often used interchangeably, and only a single quagga – the one at the London zoo – was ever photographed in life[2]. More recently, E.C. Mungal[6] argued that despite its obviously close similarity in striping pattern with the Plains zebra, the quagga's occupancy of the karroo habitat, distinct from the grassland habitat of Plains zebras, supports the impression of contemporary observers that this form was a separate species.

Conversely, the clinal variation in striping patterns of the Plains zebras thoughout their range from East Africa to the Transvaal has led other authors to consider the quagga as the extreme southern variant[3,7,8]. The two other extant species of zebras are the Mountain zebra *(Equus zebra)*, whose range includes South Africa and Namibia, and the Grevy's zebra *(Equus grevyi)*, whose range is East Africa. Because the quagga is more similar in range and pelage to the Plains zebra than to the others, the major systematic issue centers on the question whether the quagga is or is not a distinct species.

All extant species of zebras display unique karyotypes[10]. Unfortunately, we do not have the technology for doing karyotypes of extinct animals. In recent years, however, it has proved possible to extend molecular systematic studies to extinct species, notably through use of an RIA method[11].

Materials and methods. Recently specimens of several of the existing quagga skins have been made available to us for molecular analysis. We also obtained skin about 70 years old from a Plains zebra, the type specimen of *E. burchelli paucistriatus,* now synonymized with *E. burchelli burchelli.* Approximately 1 g skin was finely ground and extracted with EDTA 0.2 M. This solution was used as antigen in a solid phase RIA[11]. Additional antigens consisted of the sera of the three extant zebras, the two African ass subspecies *(E. asinus f. asinus* and *E. asinus somaliensis)*, the two Asiatic wild ass subspecies *(E. hemionus onager* and *E. hemionus kulan)* and the domestic and Przewalski's horses *(E. caballus* and *E. przewalskii)*. Antisera to these nine sera were raised in rabbits by spaced injections[11]. Direct cross-reactions

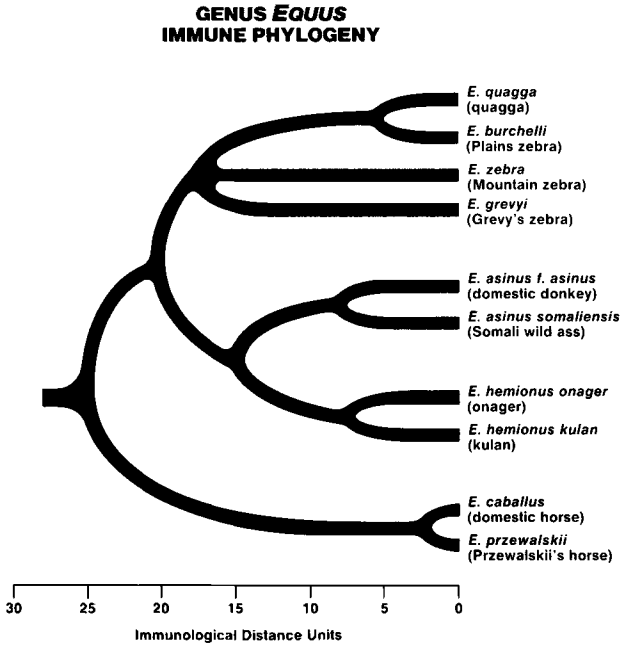
among members of the genus *Equus* are so similar that they do not distinguish individual species. These are easily separated, however, by a competitive inhibition method, in which a soluble antigen is used to inhibit the binding of antiserum to a solid phase antigen[11]. The resulting immune phylogeny is depicted in the figure.

No quagga serum was available, of course, so that placement of the quagga on this family tree was made on the basis of binding of antisera to quagga skin extracts. To accomplish this, the antisera were affinity-enhanced by passing each antiserum through columns consisting of heterologous sera bound to CnBr Sepharose (Pharmacia). In this process, antibodies common to each pair of zebras were retained on the column, and the effluent antisera were highly specific for individual species. The pattern of binding of these enhanced antisera was compared using as antigens quagga and Plains zebra skin, and sera of the three extant zebras. The similarity of quagga skin to each of the other antigens was quantified by computing correlation coefficients (r) between the amounts of the various antisera bound. Thus, when r = 1.00, the two compared antigens are identical, whereas −1.00 would indicated the maximum difference between them. A distance measure (D) can be obtained by the formula D = 100 (1−r). D = 0 for identical antigens and 200 is the maximum distance. The results are presented in the table.

Correlation coefficients (r) of immune reactions of quagga and extant zebras

	Plains skin	Quagga skin	Quagga distance (D) D = 100 (1−r)
Plains zebra skin		0.75	25
Plains zebra serum	0.95	0.72	28
Mountain zebra serum	− 0.92	− 0.40	140
Grevy's zebra serum	− 0.96	− 0.68	168

Correlation coefficients (r) are shown for the bindings by zebra skins and sera, used as solid phase antigens in radioimmunoassay (RIA), of various affinity-enhanced antisera, described in the text. Quagga skin is about 1/6 the distance (D) from Plains zebra skin and serum as from the sera of the other two extant zebras. Three different quagga skins were used and the mean values taken.



Immune phylogeny of the genus *Equus*, including the extinct quagga. Also included are the three extant zebra species, the two African ass subspecies, the two Asiatic wild ass subspecies, and the domestic and Przewalski's horses. All placements except quagga were obtained by the radioimmunoassay inhibition method[11], using whole serum as solid phase antigen and competitive inhibitor. Quagga placement was obtained as described in the text and in the legend to the table. Quagga is much closer to the Plains than to the other two zebra species, closer than the subspecies of African ass and Asiatic wild ass, but more distant than domestic from Przewalski's horse.

Results and discussion. Quagga skin is immunologically very similar to skin and serum of the Plains zebra (r = 0.75 and 0.72, respectively) and has negative correlations with Mountain and Grevy's zebra sera. The distance (D) of quagga to Plains zebra is about one-sixth that to the other two, and this result is reflected in the placement of quagga in the figure. In this immune phylogeny, the quagga to Plains zebra distance is less than that between the two donkey or the two hemionus subspecies but somewhat greater than that between the domestic and Przewalski's horses. Though they have different chromosome numbers, these horses are interfertile, and it has been questioned whether they should be considered as distinct species. We conclude that the relation between the quagga and Plains zebra is so much closer than that between the three extant zebra species that the quagga probably ought to be considered a variant of the Plains zebra and not a distinct species.

In the past two decades, molecular data have increasingly been used to construct phylogenetic trees of numerous living groups. Generally, these have been concordant with classical morphometric analysis of relationships, but in some cases the molecular affinities have disagreed with prevailing interpretations – as for instance, in showing the very close relationship between Homo and the African apes[12]. More recently, molecular systematics have been extended to extinct organisms by the RIA of fossil proteins. This approach has confirmed the close relation of the extinct mammoth *(Mammuthus primigenius)* with the living elephants and resolved a long-standing dispute over the affinites of the recently extinct Tasmanian wolf *(Thylacinus cynocephalus)* with living marsupials[11]. Here we have shown the feasibility of using fossil proteins to help decide whether an extinct form, the quagga, should be considered a distinct species or merely a variant of an extant species.

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1 Rau, R.E., Ann. S. Afr. Mus. *65*/2 (1974) 41.
2 Dolan, J.M., Zoonooz *56*/8 (1983) 13.
3 Rau, R.E., Ann. S. Afr. Mus. *77*/2 (1978) 27.
4 Harris, W.C., Portraits of the game and wild animals of southern Africa. Pickering, London 1840.
5 Ansel, W.F.H., Perissodactyla, in: Smithsonian Institution preliminary identification manual for African Mammals. Ed. J. Meester *6* (1967) 1.
6 Mungall, E.C., in: Symposium on the ecology and behavior of wild and feral equids. Ed. R.H. Denniston. Univ. of Wyoming, Laramie 1979.
7 Pocock, R.I., A. Mag. nat. Hist. *14* (1904) 313.
8 Mohr, E., Das Pelzgewerbe *15* (1964) 161.
9 Gmelin, in: Linnaeus, Syst. Nat., 13th ed. (1788) 1.
10 Ryder, O.A., Epel, N.C., and Benirschke, K., Cytogenet. Cell Genet. *20* (1978) 323.
11 Lowenstein, J.M., Sarich, V.M., and Richardson, B.J., Nature *291* (1981) 409.
12 Goodman, M., and Tashian, R.E. (Eds), Molecular Anthropology. Plenum, New York 1976.

0014-4754/85/091192-02$1.50 + 0.20/0