with private and public sector industry. Our state planning process, represented by the Planning Commission cannot deal with the R&D planning against this background since their exercise is a limited one and over the years has been reduced to a largely mechanical linear extrapolatory exercise. With the liberalization process, there is need for a large-scale indicative planning since bulk of funding for R&D in future will have to come from private industry. In turn captains of our industry will have to be sensitized to the imperative of investments in R&D. The State should be persuaded to increase incentives for such R&D investments. Yet another important area is to organize efficiently the expeditious transfer of R&D to production. Efforts will have to be made to involve the universities in a much larger scale than have so far been done.

REVIEW ARTICLE

Laboratory studies of density-dependent selection: Adaptations to crowding in *Drosophila melanogaster*

Amitabh Joshi

Evolution and Behaviour Laboratory, Animal Behaviour Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur P. O., Bangalore 560 064, India

Over the last decade or so, studies on replicated sets of *Drosophila* populations, subjected to life-stage-specific density-dependent natural selection in the laboratory, have greatly enhanced and refined our understanding regarding how these organisms adapt to crowded conditions. Fundamental trade-offs constraining the evolution of life-histories under crowded conditions have been uncovered, such as the negative association between efficiency of food acquisition and its subsequent utilization. A large suite of traits adaptive under crowded culture conditions has been identified, and the behavioural and physiological mechanisms underlying adaptation to crowding are becoming understood. These studies have yielded results that are sometimes at odds with well-established theoretical principles and have, thus, also led to a refinement of our theoretical understanding of how density-dependent selection can shape the life-histories of organisms.

THE theory of density-dependent selection represents one of the early attempts to bring population genetics and population ecology together by explicitly considering the impact that population density may have on evolutionary trends. Although the impact of density on various components of fitness had long been documented in many species[1-10], it was only in the 1960s that the basic ideas about the possible specific effects of density on life-history evolution were explicitly laid out[11,12]. The following decade saw the development of the formal mathematical theory of density-dependent selection which rapidly became one of the major components of the theory of evolutionary ecology[2-20]. A major theoretical advance of relevance to empirical biologists during this phase was the development of *Drosophila*-specific mathematical models that explicitly incorporated important aspects of the laboratory ecology of *Drosophila*[19,20]. The theoretical work done during this period clearly suggested that many of the predictions from the verbal theory of density-dependent selection[21-23] were far from accurate, because in its oversimplified verbal version, the theory had been used to explain all kinds of differences among widely different species. This part of the historical development of density-dependent selection theory has been extensively discussed elsewhere[24-27], and I will, therefore, not spend further time on it. What is more important from the point of view of this article is that the refinement of the theory gave impetus to rigorous and systematic empirical studies of density-dependent natural selection, and it is to these that I shall now turn.

Several empirical studies of density-dependent selection in the 1970s and early 1980s sought evidence cor-

roborating broad predictions of the verbal theory by comparing extant species, or conspecific populations, in the wild[21,23,28,29]. The approach used was to document differences in fitness components among these populations, and ascribe them to inferred density differences in the past. In such studies it is difficult to unequivocally attribute any observed differences to density-dependent selection because the past history of the populations is often unknown and, moreover, there are many factors beyond the control of the experimenter. A few laboratory studies of density-dependent selection were also carried out with *Drosophila* species[30–32], but these suffered from serious experimental design problems[25,27,33,34].

Around this time, a series of studies on population growth rates at different densities was initiated by F. J. Ayala and co-workers, using populations of *D. melanogaster* maintained in the laboratory[35–37]. It became clear from the somewhat equivocal results of these studies that the ideal way to test the predictions of density-dependent selection theory would be to actually rear populations at high and low densities in the laboratory, under carefully controlled conditions, and then observe their growth rates across a spectrum of densities. Density-dependent growth rates were considered the principal measure of fitness in the formal theories of density-dependent selection[13–19], and these rates are very difficult to estimate from studies on populations in their natural habitat. Such a study on six populations of *D. melanogaster* maintained in the laboratory for eight generations at either low (3 *r*-selected populations) or high density (3 *K*-selected populations) showed that the *K*-selected populations had higher population growth rates and net productivity when measured at high densities. At lower densities, the *r*-selected populations were superior, although the differences were statistically significant only in the case of net productivity[38]. This finding supported the basic idea of a trade-off between adaptation to low versus high density (the central concept underlying the theory of *r*- and *K*-selection), but was at odds with results from a similar experiment done on the bacterium *Escherichia coli*. In the case of *E. coli*, the *K*-selected populations showed higher population growth rates than their *r*-selected counterparts at all densities, high or low[39]. Why this was so is not clear, although it is likely that the *K*-selection regime used in this experiment was, in fact, resulting in strong selection for higher maximal growth rates due to the alternation of periods of growth and starvation[40].

Once it had been seen that the *r*- and *K*-selected *Drosophila* populations exhibited a density-dependent trade-off in population growth rates and productivity[38], further studies on these populations, which continued to experience density-dependent selection in the laboratory were carried out. It was seen that the *K*-selected populations had greater larval viability and adult size (a correlate of

greater female fecundity), when reared under crowded conditions as larvae[41]. At low densities, the *r*-selected populations had greater viability[41]. Although the differences between the *r*- and *K*-selected lines were greater when measured at high density, it was clear that the *K*-selected populations were adapting to high density and were, consequently, becoming somewhat inferior at lower densities, as compared to the *r*-selected lines. This observation gave rise to a series of studies that are still ongoing, in which the focus was to understand the specific behavioural and physiological mechanisms by which *K*-selected populations came to differ from their *r*-selected controls. These studies in the laboratory of Laurence Mueller, one of the early co-workers with Ayala[36,38,41] in the area of density-dependent selection, constitute the most comprehensive and rigorous empirical investigation of adaptation to crowding carried out in evolutionary ecology and will be the main focus of this review. Because the design and interpretation of these studies often depends critically on our knowledge of the laboratory ecology of crowded *Drosophila* cultures, I shall first review our understanding of this topic.

The laboratory ecology of crowded *Drosophila* cultures

The primary effects of crowding on the laboratory ecology of *Drosophila* can be grouped into several broad categories based on the life-stage at which crowding occurs and the specific aspect of the environment that is affected (Table 1). In general, the effects of larval crowding in *Drosophila* have been studied to a much greater extent than those of adult crowding[42]. The most obvious effect of larval crowding is, of course, increased intra-specific competition as a result of relatively rapid resource depletion[5,7,8]. The effects of this competition have an impact on both the larval and adult fitness[20]. One of the effects of food depletion, other than inducing higher larval mortality, is to reduce the size of the eclosing adults; smaller adult size tends to reduce future reproductive success in both the males[43] and females[44]. Knowledge of the biology of *Drosophila* larvae under food depletion also suggests possible adaptations that would help cope with larval crowding.

Drosophila larvae need to attain a critical minimum size in order to successfully pupate[5,8]. This, in turn, implies that there is a minimum food requirement for successful pupation, the magnitude of which would be a reflection of the intrinsic 'efficiency' of the larva in converting food to biomass. Individuals with a relatively lower minimum food requirement may, thus, be expected to have higher fitness under crowded conditions as they will be able to complete development on relatively smaller amounts of food; indeed, this was one of the predictions made by MacArthur and Wilson[12] for

Table 1. The range of phenotypic effects caused by crowding in laboratory cultures of *D. melanogaster*. Some of these effects are well established; others (marked by asterisks) may be seen only under specific environmental conditions or are putative, supported by only preliminary evidence

Life-stage at which crowding occurs	Primary effect (ecological consequence) of crowding	Secondary (phenotypic) effects of crowding	
		On larvae	On adults
Larvae	Food limitation due to depletion	Increased pre-adult mortality and development time	Decreased body size and, therefore, fecundity
	Build-up of nitrogenous wastes		
	i) exposure as larvae	Increased pre-adult mortality and development time	Decreased body size, fecundity and longevity
	ii) exposure as adults	–	Decreased fecundity, increased longevity
	Medium becomes extremely soggy and moist* i) exposure as larvae*	Increased mortality of pupae on the surface of the medium	–
	ii) exposure as adults*	–	Increased mortality of adults coming to the medium surface
Adults	Food limitation	–	Reduced fecundity, longevity
	Medium becomes soggy and moist	–	Increased mortality of adults coming to the medium surface
	Crowding *per se* (increased interactions with conspecifics)*	–	Reduced fecundity*

k-selected organisms. Another possible strategy that populations experiencing crowding may evolve is to become faster feeders and more active foragers as larvae. Larval feeding rate, measured as the number of cephalopharyngeal sclerite retractions per minute[45], is a largely additive trait that responds to artificial selection in both directions[45,46], and is known to be correlated with competitive ability[45-47]. The extent of locomotory activity of larvae while foraging is known to be primarily determined by a single locus *for*, with increased locomotion (rover phenotype) being dominant[48-53]. The genetic control of larval digging activity (the depth to which larvae tend to burrow into the medium) has also been established: the trait is quantitative with substantial additive and dominance components, the latter being for increased digging[54,55]. Increased locomotion and digging while feeding, may help dispersal of larvae in three dimensions, thereby ameliorating the severity of competition in a crowded culture.

Another problem that affects both the larvae and ovipositing females in a crowded culture is the build-up over time of fairly high levels of toxic nitrogenous metabolic wastes like urea, uric acid and ammonia[56,57]. Larval biotic residues are known to inhibit both larval survivorship[58,59] and female fecundity[60]. Exposure to urea has also been shown to reduce survival and slow down the development of *Drosophila* larvae[56,57], as well as reduce subsequent adult size[27]. The presence of urea in the medium also inhibits female fecundity[61], and this effect has both short-term behavioural and longer-term cumulative (possibly physiological) components (A. Joshi, W. A. Oshiro, J. Shiotsugu and L. D. Mueller, unpubl. data). Increased larval tolerance to the deleterious effects of urea can be successfully selected for[62] and is known to be a largely dominant trait and affected

strongly by both X-linked loci and maternal effects[63]. Much of the work on tolerance to nitrogenous wastes has been done using urea. However, recent work suggests that urea may not be the primary nitrogenous waste product in *Drosophila* cultures (D. J. Borash, A. G. Gibbs and L. D. Mueller, unpubl. data). It is not known whether, or to what extent, tolerance to different nitrogenous waste products is genetically correlated. Nevertheless, it is clear that tolerance to relatively high levels of nitrogenous wastes is likely to be selectively advantageous in crowded *Drosophila* cultures.

Yet another aspect of crowded *Drosophila* cultures, that may impact fitness in both pre-adult and adult phases, is the consistency of the medium. In a culture that contains a high density of larvae or adults, the food medium rapidly becomes very moist and soggy. In such cultures, individuals pupating on or close to the surface of the medium have an increased chance of being dislodged and drowned in the medium[64]. Similarly, the adults in an extremely crowded culture suffer high levels of mortality through drowning in the medium; this effect is more pronounced in females[42], presumably because they need to go to the surface of the medium to feed and oviposit whereas *Drosophila* males feed very little.

Compared to larval crowding, the effects of adult crowding in laboratory cultures of *Drosophila* are not very well known. Brief episodes (3–5 days) of crowding early in adult life, in addition to increasing mortality through drowning, also lower the subsequent fecundity and longevity of the survivors[42,65]. There is some preliminary evidence that this decrease in fecundity and longevity may be due to reduced accumulation of lipids (A. Joshi, W. Wu and L. D. Mueller, unpubl. data). Generally, in the first few days after eclosion, *Droso-*

phila females put on weight[42] and may be building up lipid reserves for egg production. Apparently, crowding during this time may reduce the ability of the females to build up these lipid reserves, although the evidence for this is still extremely preliminary. Another well-known effect of adult density in *Drosophila* is the reduction of female fecundity: fecundity declines almost hyperbolically with increasing adult density[1,5,66], although the steepness of the decline can be reduced by high food levels[20,25,27,66]. Moreover, this decline in fecundity is at least partly the consequence of increased interactions among adults and is not merely due to decreased per capita food availability at high densities[1,25].

Crowding and food levels during the adult and larval phases can also have significant effects on the population dynamics of *Drosophila* cultures. A detailed model of *Drosophila* population dynamics incorporating density-dependent viability of the larvae and the effects of larval crowding and adult crowding on female fecundity, predicted that population stability would be especially sensitive to the manner in which fecundity responds to adult density: a relatively rapid decline in fecundity with increasing density would tend to generate stability and vice versa[20]. This model has been shown to adequately account for the observed effects of larval crowding on pre-adult viability and adult size[67]. The prediction about population stability has also been verified empirically by showing that populations given low levels of food in the larval phase and excessive food in the adult phase (to reduce the sensitivity of fecundity to density) exhibit large oscillations in population size from one generation to the next[66]. Experimental validation of several key predictions of this model suggests that our understanding of the major factors affecting the ecology of crowded *Drosophila* cultures is reasonably good.

Adaptation to crowding in *D. melanogaster* under laboratory natural selection

From the foregoing discussion of the manifold phenotypic effects of crowding in *Drosophila*, it should be clear that there are many potential ways in which populations can adapt to crowding and enhance their fitness under high-density conditions. At the same time, it is unlikely that a population could simultaneously evolve all, or most, of the possible adaptations to crowding as a consequence of the ubiquity of life-history trade-offs that preclude the simultaneous maximization of many components of fitness. The evolution of adaptations to crowding in replicated sets of *D. melanogaster* populations, subjected to density-dependent natural selection in the laboratory, has been extensively studied over the past decade or so by Laurence Mueller and his co-workers, and this work has given us many insights into the trade-offs constraining density-dependent evolution

in this species, as well as an appreciation of the subtle influences of rearing environment on responses to selection for increased adaptation to crowding (Table 2). Most of these studies have been carried out on two separate sets of populations: the six *r*- and *K*-populations established by Mueller and Ayala[36], referred to earlier, and, more recently, three groups of five replicate populations each, subjected to varying levels of larval and adult crowding (the UU, UC and CU pouplations described by Mueller *et al.*[68] and Joshi and Mueller[69]. UU lines are controls, *u*ncrowded both as larvae and as adults, UC lines are *u*ncrowded as larvae but *c*rowded as adults, and CU lines are *c*rowded as larvae and *u*ncrowded as adults).

Larval feeding rate versus efficiency at converting food to biomass

One behavioural adaptation that has repeatedly been observed to evolve in populations maintained at high larval density for many generations is an increased larval feeding rate[46,69,70]. Larvae from populations regularly kept at high larval densities typically take up to 20–30 bites more per minute than larvae from unselected populations maintained at low larval densities[47,69]. These larvae also have increased competitive ability against tester stocks, compared to larvae from unselected control populations[71]. More interestingly, there is evidence from both systems (*r*- and *K*-populations as well as CU and UU populations) that this increased feeding rate comes at an energetic cost: larvae from the crowded populations that feed faster than those from the control populations, actually require more food than the control larvae to successfully complete development[69,72]. This is contradictory to predictions from both the verbal[12] and mathematical[20] theories of density-dependent selection which predict that evolution in a crowded environment should favour increased efficiency of conversion of food to biomass. This finding is further corroborated by the observation that populations that have evolved higher larval feeding rates after being subjected to larval crowding for over 50 generations undergo a rapid (within 15 generations) decline in their feeding rates when they are maintained at a moderately low larval density[70]: such a decline upon relaxation of selection is indicative of a cost to higher feeding rates.

It is possible to argue that, in principle, larvae from crowded populations could still be more 'efficient' in utilizing food, despite their higher minimum food requirement for pupation, by either eclosing as heavier adults, or by completing development faster than the larvae from control populations. Both these simple explanations for the greater minimum food requirement for pupation shown by larvae from crowded populations have been ruled out by subsequent experiments on the

Table 2. The suite of adaptations seen to evolve in populations of *D. melanogaster* maintained at high densities in the laboratory. Some of these adaptations are well established; others (marked by asterisks) may be seen only under specific environmental conditions or are putative, supported by only preliminary evidence. Where the genetic control of the trait is known, it is summarized in parentheses (Q, quantitative, S, simple Mendelian, A, largely additive, D, major dominance component, X, X-linked, ME, maternal effects)

Life-stage at which crowding occurs	Primary effect (ecological consequence) of crowding	Traits seen to evolve under high-density	
		In larvae	In adults
Larvae	Food limitation due to depletion	Increases in larval feeding rate (Q, A), foraging activity (S, D), growth rate during active feeding larval phase*, and weight loss during post-feeding larval phase*	Increases in ability to store lipids and glycogen*, and in locomotor activity (S, D)*
	Build-up of nitrogenous wastes (exposure as larvae)	Increased larval tolerance to the toxic effects of urea (Q, D, X, ME)	No change in adult susceptibility to urea
	Medium becomes extremely soggy and moist (exposure as larvae)*	Increased height above medium at which larvae pupate*	–
Adults	Food limitation*	–	Reduced susceptibility to deleterious effects on fecundity and longevity induced by an episode of crowding early in adult life
	Medium becomes soggy and moist	–	Reduced mortality at medium surface, possibly through selection for negative geotaxis*

CU and UU populations[73]. In these experiments, larvae from crowded (CU) populations were seen to have faster feeding rates and higher wet weights compared to the controls (UU), especially during the third instar of larval development after the attainment of the minimum critical size for pupation[73]. However, the increased weight of late third instar larvae from the CU populations did not translate into increased adult weight; eclosing adults from both the CU and UU populations did not differ in wet weight. The development times of larvae from the CU and UU populations also did not significantly differ from one another[73]. Another possible explanation for the greater minimum food requirement for pupation of the CU populations could be that faster passage of food through the gut, as a consequence of faster feeding, results in incomplete absorption of nutrients. This possibility has been ruled out by showing that the time taken for food to pass through the gut of larvae from the CU and UU populations is not significantly different[69].

The results on the time course of larval feeding rate and weight gain in the CU and UU populations tend to support the suggestion made by Joshi and Mueller[69] that the CU larvae are less efficient at converting food to biomass because of the energetic cost of higher feeding rates and increased larval activity. The CU larvae have higher locomotory activity than their UU counterparts (they have mostly rover phenotypes)[74], and the greatest expression of the rover phenotype is late in the third instar[75]. It appears that the CU larvae lose their weight advantage, gained by faster feeding, over the UU larvae during the post-feeding phase prior to pupation, late in

the third instar. Density-dependent natural selection in crowded cultures, thus, seems to favour efficiency in obtaining food at the expense of efficient conversion of the ingested food to biomass.

Larval tolerance to urea

Studies on the CU and UU populations have also shown that the CU populations have evolved greater tolerance to the toxic effects of urea. Compared to their UU counterparts, CU larvae have significantly greater survivorship and faster development on medium with 18 g/l of urea (a highly toxic concentration)[62]. Not surprisingly, the rate at which nitrogenous wastes accumulate in the food medium is much higher in CU cultures than in the uncrowded UU cultures (D. J. Borash, A. G. Gibbs and L. D. Mueller, unpubl. data). The mechanism by which the CU larvae are able to tolerate these high levels of urea is not yet known.

Pupation height

Pupation height, the height above the surface of the medium at which larvae pupate, offers a good example of how environmental factors can modulate responses to selection through genotype–environment interactions, which are, of course, ubiquitous in life-history evolution[76]. Mueller and Sweet[77] observed that the pupation height of the larvae from K-selected populations was greater than their r-selected controls. At that time it

seemed reasonable to assume that the medium in the crowded K-selected cultures became very soggy and caused increased mortality of the individuals pupating on the surface of the medium, thereby selecting for increased pupation height. A more detailed study of mortality of individuals pupating at different heights above the medium in both the r- and K-populations, however, showed that even at low densities, selection should favour increased pupation height because individuals pupating on the food surface suffered extremely high mortality even at densities as low as 20 larvae per 8-dram vial[64]. When similar studies were repeated on the CU and UU populations, initially the CU populations evolved greater pupation heights than the UU controls[69]. After about 60 generations of selection, however, the difference in pupation height between the CU and UU populations was no longer discernible[69].

The explanation for these mutually inconsistent results may lie in the details of the maintenance regimes of the CU, UU, r- and K-populations and the conditions under which pupation height was assayed. It has been suggested that because the banana-molasses food used for the CU and UU populations was intrinsically softer and more moist than the cornmeal food used for the r- and K-populations, the UU populations were also experiencing selection for increased pupation height, albeit less strongly than the more crowded CU populations[69]. Since selection for the increased pupation height in the crowded cultures is of the stabilizing type[64], the CU populations reached a selection plateau earlier; after about 60 generations, the UU populations had also reached the selection plateau and the difference in pupation height between the two types of population was, therefore, no longer observed[69]. The r-populations were maintained in milk-bottles with a facial tissue inserted to control moisture. In these cultures, most larvae tended to pupate on the tissue rather than on the food or on the sides of the culture bottle. It has been postulated that the r-selected larvae, consequently, fortuitously escaped selection for increased pupation height[64]. The mortality of different pupation height phenotypes, however, was measured in vials without any facial tissue; thus, even at low assay densities, the larvae pupating on the surface of the medium suffered increased mortality[64].

Adaptation to adult crowding

Recent studies comparing the effects of adult crowding on the CU, UC and UU populations have provided clear evidence for the evolution of specific adaptations to adult crowding in the UC populations that are maintained at high adult densities. The behavioural and/or physiological mechanisms underlying this adaptation, however, are as yet not clear. The UC populations have been seen to suffer significantly less mortality than ei-

ther the UU or CU populations, when subjected to 3–5 days of extreme crowding early in adult life[42]. The degree to which such an episode of crowding reduces subsequent longevity and female fecundity is also the least in the UC populations[42,65]. There is some preliminary evidence that the UC populations may have evolved a reduced propensity for the adults to go down to the surface of the medium during early adult life[42], drowning in the soggy medium is the major cause of mortality during adult crowding. More interestingly, it has been seen that the CU populations that are adapted to high larval density, are actually more susceptible to the deleterious effects of adult crowding than even the UU controls[42,65], raising the intriguing possibility of trade-offs between adaptations to larval versus adult crowding.

Conclusions

Particular areas of research within any scientific discipline seem to drift in and out of fashion in much the same way as specific hair styles or notions of what constitutes sartorial elegance. In the 1970s density-dependent selection occupied pride of place in evolutionary ecology. Shortly thereafter, it suffered a massive decline in popularity and acceptability, largely through the sloppy work of some researchers who tried to explain everything under the sun in terms of differences in density, and retreated into relative oblivion. Since then, however, our understanding of the density-dependent evolution of life-history traits in *Drosophila* cultures has been vastly enriched by the studies discussed here. Indeed, the main message from many of these studies would appear to be that the action of density-dependent natural selection can be much more subtle and unexpected than was earlier thought to be the case.

Today, we know a great deal about the behavioural and physiological adaptations to crowding in *Drosophila*[27,63,69,78–80]. The reality of a trade-off between efficiency of larval food acquisition and utilization is well established. There is clearly much more to be learned about the physiological underpinnings of this crucial trade-off. Studies on the metabolic rates of larvae from populations adapted to crowding, though technically daunting, should prove very fruitful. The physiological mechanisms of urea tolerance are still a mystery. Similarly, the energetic cost of such tolerance is yet to be determined; it is highly likely that the cost will be nontrival. There is tantalizing preliminary evidence that trade-offs btween adaptation to larval and adult crowding may exist[42,65]. Such trade-offs are likely to be moored in the physiological network connecting nutrient intake, assimilation and storage with primary biological attributes such as growth rates and the allocation of resources between reproduction and somatic maintenance. There is a possibility of multiple suites of strategies to

cope with crowding in *Drosophila* and it may be that populations adapted to crowding are actually polymorphic for such adaptive suites of traits (D. J. Borash, A. Joshi, and L. D. Mueller, unpubl. data). I certainly hope that density-dependent selection, like most fashions, will return to the limelight, for there is much more to be studied in this most interesting phenomenon which, although presently unfashionable, is certainly not unimportant to evolutionary ecology.

I hope that this review has also served to underscore the fact that natural selection in the laboratory on replicated sets of populations constitutes a very powerful system for the elucidation of evolutionary trends and trade-offs[27,69,81–83]. No doubt, such studies do not address the question of what actually happens in nature. Nevertheless, they sharpen and refine our appreciation of what might and what might not be possible in evolution and, moreover, permit a dissection of the mutually inter-linked components of life-history evolution under specific ecological scenarios in a manner quite impossible under the relatively uncontrollable conditions of the field.

1. Pearl, R., Miner, J. R. and Parker, S. L., *Am. Nat.*, 1927, **61**, 289–318.
2. Park, T., *Ecology*, 1932, **13**, 172–181.
3. Utida, S., *Memoirs Coll. Agri. Kyoto. (Entomol. Series 8)*, 1941, **51**, 1–26.
4. Davis, M. B., *Ecology*, 1945, **26**, 353–362.
5. Chiang, H. C. and Hodson, A. C., *Ecol. Monogr.*, 1950, **20**, 173–206.
6. Frank, P. W., Bell, C. D. and Kelly, R. W., *Physiol. Zool.*, 1957, **4**, 287–305.
7. Bakker, K., *Entomol. Exp. Appl.*, 1959, **2**, 171–186.
8. Bakker, K., *Arch. Neerl. Zool.*, 1961, **14**, 200–281.
9. Tanner, J. T., *Ecology*, 1966, **45**, 733–745.
10. Bakker, K., *Neth. J. Zool.*, 1969, **19**, 541–595.
11. MacArthur, R. H., *Proc. Natl. Acad. Sci. USA*, 1962, **48**, 1893–1897.
12. MacArthur, R. H. and Wilson, E. O., *The Theory of Island Biogeography*, Princeton University Press, Princeton, 1967.
13. Charlesworth, B., *Ecology*, 1971, **52**, 469–474.
14. King, C. E. and Anderson, W. W., *Am. Nat.*, 1971, **105**, 137–156.
15. Clarke, B., *Am. Nat.*, 1972, **106**, 1–13.
16. Roughgarden, J., *Theory of Population Genetics and Evolutionary Ecology: An Introduction*, MacMillan, New York, 1979.
17. Iwasa, Y. and Teramoto, E., *J. Theor. Biol.*, 1980, **84**, 545–566.
18. Asmussen, M. A., *Genetics*, 1983, **103**, 335–350.
19. Nunney, L., *Am. Nat.*, 1983, **121**, 67–93.
20. Muller, L. D., *Am. Nat.*, 1988, **132**, 786–809.
21. Pianka, E. R., *Am. Nat.*, 1970, **104**, 952–956.
22. Pianka, E. R., *Am. Nat.*, 1972, **106**, 581–588.
23. Gadgil, M. and Solbrig, O. T., *Am. Nat.*, 1972, **106**, 14–31.
24. Boyce, M. S., *Annu. Rev. Ecol. Syst.*, 1984, **15**, 427–447.
25. Mueller, L. D., *Evol. Biol.*, 1985, **19**, 37–98.
26. Travis, J. and Mueller, L. D., in *Blending Ecology and Genetics: Progress Toward a Unified Population Biology* (eds Roughgarden, J., May, R. M. and Levin, S. A.), Princeton University Press, Princeton, 1989, pp. 101–124.
27. Mueller, L. D., in *Genetics of Natural Populations: The Continuing Importance of Theodosius Dobzhansky* (ed. Levine, L.), Columbia University Press, New York, 1995, pp. 101–124.
28. Abrahamson, W. G. and Gadgil, M., *Am. Nat.* 1973, **107**, 651–661.
29. McNaughton, S. J., *Am. Nat.*, 1975, **109**, 251–261.
30. Taylor, C. E. and Condra, C., *Evolution*, 1980, **34**, 1183–1193.
31. Barclay, H. J. and Gregory, P. T., *Am. Nat.*, 1981, **117**, 944–961.
32. Barclay, H. J. and Gegory, P. T., *Am. Nat.*, 1982, **120**, 26–40.
33. Stearns, S. C., *Q. R. Biol.*, 1976, **51**, 3–47.
34. Stearns, S. C., *Annu. Rev. Ecol. Syst.*, 1977, **8**, 145–171.
35. Tracey, M. L. and Ayala, F. J., *Genetics*, 1974, **77**, 569–589.
36. Mueller, L. D. and Ayala, F. J., *Genetics*, 1981, **97**, 667–677.
37. Seager, R. D. and Ayala, F. J., *Genetics*, 1982, **102**, 467–483.
38. Mueller, L. D. and Ayala, F. J., *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 1303–1305.
39. Luckinbill, L. S., *Science*, 1978, **202**, 1201–1203.
40. Vasi, F., Travisano, M. and Lenski, R. E., *Am. Nat.*, 1994, **144**, 432–456.
41. Bierbaum, T. J., Mueller, L. D. and Ayala, F. J., *Evolution*, 1989, **43**, 382–392.
42. Joshi, A., Wu, W. and Mueller, L. D., *Evol. Ecol* (in press).
43. Wilkinson, G. S., *Evolution*, 1987, **41**, 11–21.
44. Mueller, L. D., *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 1974–1977.
45. Sewell, D., Burnet, B. and Connolly, K., *Genet. Res.*, 1975, **24**, 163–173.
46. Burnet, B., Sewell, D. and Bos, M., *Genet. Res.*, 1977, **30**, 149–161.
47. Joshi, A. and Mueller, L. D., *Evolution*, 1988, **42**, 1090–1093.
48. Bauer, S. J. and Sokolowski, M. B., *Can. J. Genet. Cytol.*, 1985, **27**, 334–340.
49. Bauer, S. J. and Sokolowski, M. B., *Behav. Genet.*, 1988, **18**, 81–97.
50. De Belle, J. S. and Sokolowski, M. B., *Heredity*, 1987, **59**, 73–83.
51. De Belle, J. S., Hilliker, A. J. and Sokolowski, M. B., *Genetics*, 1989, **123**, 157–163.
52. De Belle, J. S., Sokolowski, M. B. and Hilliker, A. J., *Genome*, 1993, **36**, 94–101.
53. Pereira, H. S., MacDonald, D. E., Hilliker, A. J. and Sokolowski, M. B., *Genetics*, 1995, **141**, 263–270.
54. Godoy-Herrera, R., *Heredity*, 1986, **56**, 33–41.
55. Godoy-Herrera, R., *Behav. Genet.*, 1994, **24**, 427–432.
56. Botella, L. M., Moya, A. and Mensua, J. L., *Dros. Inf. Serv.*, 1983, **59**, 23–24.
57. Botella, L. M., Moya, A., Gonzalez, M. C. and Mensua, J. L., *J. Insect. Physiol.*, 1985, **31**, 179–185.
58. Budnik, M. and Brncic, D., *Ecology*, 1974, **55**, 657–661.
59. Budnik, M. and Brncic, D., *Evolution*, 1976, **29**, 777–781.
60. Aiken, R. B. and Gibo, D. L., *Oecologia*, 1979, **43**, 63–77.
61. Joshi, A., Shiotsugu, J. and Mueller, L. D., *Exp. Gerontol.*, 1996, **31**, 533–544.
62. Shiotsugu, J., Leroi, A. M., Yashiro, H., Rose, M. R. and Mueller, L. D., *Evolution*, 1997, in press.
63. Joshi, A., Knight, C. D. and Mueller, L. D., *Heredity*, 1996, **77**, 33–39.
64. Joshi, A. and Mueller, L. D., *Evolution*, 1993, **47**, 176–184.
65. Joshi, A. and Mueller, L. D., *Curr. Sci.*, 1997, **72**, 255–260.
66. Mueller, L. D. and Huynh, P. T., *Ecology*, 1994, **75**, 430–437.
67. Mueller, L. D., Gonzalez-Candelas, F. and Sweet, V. F., *Am. Nat.*, 1991, **137**, 457–475.
68. Mueller, L. D., Graves, J. L. and Rose, M. R., *Func. Ecol.*, 1993, **7**, 469–479.
69. Joshi, A. and Mueller, L. D., *Evol. Ecol.*, 1996, **10**, 463–474.
70. Joshi, A., Castillo, R. B. and Mueller, L. D., unpubl. ms.
71. Mueller, L. D., *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 4383–4386.
72. Mueller, L. D., *Evol. Ecol.*, 1990, **4**, 290–297.

73. Santos, M., Borash, D. J., Joshi, A., Bounlutay, N. and Mueller, L. D., *Evolution*, 1997, in press.
74. Sokolowski, M. B., Pereira, H. S. and Hughes, K., *Genetics*, 1997, in press.
75. Graf, S. A. and Sokolowski, M. B., *J. Insect Behav.*, 1989, **2**, 301–313.
76. Leroi, A. M., Chippindale, A. K. and Rose, M. R., *Evolution*, 1994, **48**, 1244–1257.
77. Mueller, L. D. and Sweet, V. F., *Evolution*, 1986, **40**, 1354–1356.
78. Mueller, L. D., *Philos. Trans. R. Soc. London*, 1991, **B332**, 25–30.
79. Mueller, L. D., Guo, P. Z. and Ayala, F. J., *Science*, 1991, **253**, 433–435.
80. Guo, P. Z., Mueller, L. D. and Ayala, F. J., *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 10905–10906.
81. Fleming, J. E., Spicer, G. S., Garrison, R. C. and Rose, M. R., *Genetica*, 1993, **91**, 183–198.
82. Joshi, A. and Thompson, J. N., *Evolution*, 1997, in press.
83. Joshi, A., Oshiro, W. A., Shiotsugu, J. and Mueller, L. D., *J. Biosci.*, 1997, in press.

RESEARCH ARTICLE

Anisotropy across biological membranes: Histidine charges oppose net charge anisotropy

V. Sitaramam

Department of Biotechnology, University of Pune, Pune 411 007, India

Considerable interest exists to find unique means to define and assess the inhomogeneity of the transmembrane terrain in terms of structural components such as the properties of the transmembrane proteins as an efficient explanation for vectorial transport, i.e. transport in specified direction. A number of properties of amino acids exhibit significant transmembrane anisotropy, i.e. directionality, when normalized per residue of the aqueous loops. Charge anisotropy in terms of fixed charges was reinvestigated and while the 'positive inwards' rule is obeyed grossly, classes of proteins were seen to show significant distinction among themselves such that receptors exhibit anisotropy 'positive inwards' while ATPases do not. Most interestingly, histidine exhibits transmembrane anisotropy opposite in sign to charge anisotropy among all classes of membrane proteins thus far examined.

DISPOSITION of proteins and amino acids across biological membranes is not symmetric. Certain aspects of this asymmetry are functionally obvious: the receptor proteins with external binding sites would have a larger bulk of the protein facing externally while transport ATPases would have the bulkier active site facing the cytoplasmic aspect. In specific instances, such an anisotropy lends clues to the function, e.g. charge anisotropy of membrane proteins[1]. Charge disposition among membrane proteins was originally described by Von Heijne as 'positive inside' rule which led to interesting possibilities on membrane biosynthesis and insertion of proteins into the membranes[2-4]. A more detailed quantitative analysis significantly amplifying this observation showed that the significant variable is positive inwards and not merely positive inside, based on the available data of confirmed and putative aqueous loops[1]. The direction was consistent with a functional role for this anisotropy such as the direction of proton movements: protons tend to move from the more positive side to the more negative side in biological membranes, which gave the physical basis of Mitchell's conjecture[5] that protons would move vectorially at least in the sense of a fixed direction. The directionality of the pump varies with whether the pump is the primary, the gradient generator, or the secondary, the gradient dissipator. Thus, the secondary pumps should have opposite anisotropy[5,6], do they?

Indeed several specific questions surfaced as a consequence of the above observations: (i) There being a large number of proteins now identified with putative predicted transmembrane topology, does the positive 'inwards' rule remain valid with a much larger sample? (ii) More interestingly, the distribution of internal and