

malian DNase I and *Escherichia coli* CdtB.

DNase I proteins—enzymes that cut DNA into smaller pieces—share conserved amino acid residues that are important for enzyme activity. Both groups (1, 2) tested mutant CdtB proteins containing altered conserved residues to see whether the toxins were still potent. Each mutation resulted in a substantial decrease in CdtB toxicity. Elwell and Dreyfus (2) correlated the decrease in toxicity with a concomitant decrease in DNase I activity in vitro. Meanwhile, Lara-Tejero and Galán (1) showed that CdtB became localized in the nucleus of toxin-treated cells, consistent with its proposed role as a DNase (1).

Identification of CDT as a DNase immediately suggests a model for how the toxin arrests the host cell in G₂. Damage to the DNA induces cell cycle arrest by triggering signaling cascades that keep Cdc2, a key regulatory protein, in an inactive (phosphorylated) form (5) (see the figure). Damage to the DNA inflicted by CDT results in activation of a damage response pathway, and accumulation of inactive Cdc2 resulting in arrest of cells in G₂ (4). Continued biosynthesis by these arrested cells may result in the characteristic distension of the cytoplasm associated with CDTs. DNA damage inflicted by CdtB results in chromatin fragmentation and eventual cell death.

Whereas DNA damage normally results in arrest of cells at either G₁ (just before DNA replication) or G₂ (just before cell division), CDT-treated cells invariably halt in G₂ only. Lara-Tejero and Galán propose that CdtB damages DNA only when the DNA is in a vulnerable physical state, that is, during replication in S phase. Indeed, exposure of cells to CDT during DNA replication is required for arrest at the subsequent G₂; exposure to CDT after DNA replication is complete allows cells to progress through mitosis and not to halt until the next G₂ (see the figure) (4).

An unusual aspect of the CDT family is that its members are made by diverse sorts of bacteria. The only common feature of all known CDT-producing bacteria is that they infect epithelial cell layers, such as those comprising the gastrointestinal or genitourinary tract. Epithelial cells would be especially sensitive to the cell cycle-arresting activity of CDTs, because they continuously proliferate and differentiate as they migrate from deeper layers toward the epithelial surface, from which they are eventually shed. Disruption of normal epithelial cell turnover could lead to breakdown of the epithelial barrier, permitting easier access of bacteria and their secreted toxins to underlying tissues. Cells of the immune system are another potential tar-

get for CDTs because they proliferate in response to antigen. Supporting this hypothesis is the finding that certain CDTs inhibit proliferation of monocytes and lymphocytes (3, 6), potentially affecting both innate and acquired immunity.

Now that CDTs have been identified as DNases, many exciting avenues for investigation should open up. For example, a next step will be to identify the host cell or bacterial factors that regulate the DNase activity of CDTs or that deliver CdtB to the nucleus (perhaps with the help of the other two CDT subunits). Further amino acid mutation studies will allow rigorous analysis of the importance of CdtB's DNase activity in animal models of infection. With the finding that CDT family members are DNases, pathogenic bacteria have provided us with yet more tools to study basic biological processes in the eukaryotic cell—in this case, control of the cell cycle.

References

1. M. Lara-Tejero and J. E. Galán, *Science* **290**, 354 (2000).
2. C. A. Elwell and L. A. Dreyfus, *Mol. Microbiol.* **37**, 952 (2000).
3. C. L. Pickett and C. A. Whitehouse, *Trends Microbiol.* **7**, 292 (1999).
4. C. Comayras *et al.*, *Infect. Immun.* **65**, 5088 (1997); S. Y. Peres *et al.*, *Mol. Microbiol.* **24**, 1095 (1987).
5. G. P. Dasika *et al.*, *Oncogene* **18**, 7883 (1999).
6. V. Gelfanova, E. J. Hansen, S. M. Spinola, *Infect. Immun.* **67**, 6394 (1999); B. J. Shenker *et al.*, *J. Immunol.* **162**, 4773 (1999).

PERSPECTIVES: EVOLUTION

The Benefits of Allocating Sex

Stuart A. West, Edward Allen Herre, Ben C. Sheldon

Evolutionary biologists have developed an excellent understanding of the selective factors that shape the way in which a given organism allocates resources to male and female offspring—a process called sex allocation (1). Studies of sex allocation have provided explanations for a wide range of phenomena—for example, the variation among animals in the proportion of offspring that are male (sex ratio), pollen-ovule ratios in plants, and the age of sexual transition in organisms such as certain coral reef fish that change sex during their lifetime (1, 2). The strength of empirical support for the existence of sex allocation and the selective factors that shape it allows studies of sex allocation to address more detailed questions about natural selection. Furthermore, sex allocation theory can be used to elucidate the pop-

ulation structure and epidemiology of medically important pathogens such as the protozoan parasites that cause malaria.

Precision of Adaptation

At a time when school boards in the United States are debating whether to include the theory of evolution by natural selection as part of the curriculum, studies of sex allocation are providing some of the best support for this theory (2). There are several reasons for this: (i) sex allocation can often have a clear, immediate, and direct effect on fitness; (ii) theoretical models are based on relatively simple trade-offs that often rely on only a small number of key variables; and (iii) the important variables are usually easy to measure. Moreover, relative to most other traits, sex allocation has the advantage that predictions of optimal allocation patterns can be derived from first principles that are directly linked to the most basic elements of evolutionary theory.

For example, extreme sex ratio adjustments in fig-pollinating wasps confirm

many of the tenets of evolutionary theory (3–5). There are many species of fig-pollinating wasps, and in each case, female wasps pollinate and lay eggs in the enclosed fruit of their own host fig species. Mating occurs between the wasps that develop in the same fruit, before the females disperse. Typically, if only a single female lays eggs in a fruit, she produces an extremely female-biased sex ratio (only 5 to 10% of the offspring are males). As the number of females laying eggs in a fruit increases, the sex ratios in the broods become less biased (see the figures, next page, top and bottom). Although there are deviations between observed sex ratios and those predicted by theory (6), the fit is often very close.

The observed deviations from the predicted optimal sex ratio are not random. When different fig-pollinating wasp species are compared, the mean sex ratio of offspring produced by a given number of females laying eggs in a fruit is closest to theoretical predictions for the situations (number of females laying eggs in a fruit) that are encountered most frequently in that species (4). Furthermore, females show a greater ability to alter their brood sex ratios (in response to variations in the number of females laying eggs in the same fruit) in those species where the number of females enter-

S. A. West is at the Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, UK. E-mail: stu.west@ed.ac.uk E. A. Herre is at the Smithsonian Tropical Research Institute, Republic of Panama. B. C. Sheldon is in the Department of Zoology, University of Oxford, Oxford OX1 3PS, UK.

ing a fruit is more variable (4) (see the figure, next page, top). Finally, females show less variation in the sex ratio of their broods in the situations (number of females laying eggs in a fruit) that they encounter most frequently (5). Although these data provide strong evidence for adaptive sex allocation, they also suggest that organisms vary in the degree to which their behavior fits theoretical optima.

Sex allocation offers excellent opportunities for examining the constraints and limits on adaptation. Unfortunately, the best understood constraints on adaptation tend to be striking developmental or phylogenetic examples that are of limited general application. Very little is known about factors limiting adaptation that could be applied to most organisms (7), such as mutation, antagonistic pleiotropy (genes that improve one aspect of adaptation while reducing another), and processing of information from the environment.

The most striking sex ratio patterns have been found in insects, especially the Hymenoptera (ants, bees, wasps). The haplodiploid genetic system of these insects allows females to control the sex of offspring by regulating whether eggs are fertilized or not—males are haploid (single set of chromosomes) and develop from unfertilized eggs, whereas females are diploid (double set of chromosomes) and develop from fertilized eggs. In contrast, vertebrates rarely exhibit extreme skews in sex ratio, which may reflect a constraint imposed by chromosomal sex determination (1). However, recent studies of mammals and particularly birds (for example the Seychelles warbler) have shown some striking shifts in the sex ratio of offspring (8), suggesting that, contrary to popular assumptions, genetic sex determination is not an all-powerful constraint on sex allocation.

There are alternative explanations for why fewer cases of extreme sex ratio skews exist in vertebrates. A fig wasp may be able to assess the number of females currently laying eggs in the same fruit more easily than a mammal can assess factors influencing sex allocation, such as the amount of lactation that she will be able to provide or the genetic quality of her mate (9). Extreme shifts in vertebrate sex ratios may represent cases where variables can be assessed reliably. For example, among Seychelles warblers the variable is the quality of territory, which is determined by the availability of their food source (insects). The daughters of warbler offspring help their parents rear

subsequent progeny, whereas the sons disperse. In high-quality territory, having a helper is advantageous and so predominantly daughters are produced, whereas in low-quality territory the increased competition for food means that a helper is a disadvantage and so mainly sons are produced. Another complication for vertebrates is that the combination of factors influencing sex ratio can be complex. This complexity decreases the selective advantage of shifting the sex ratio in response to any single factor. In most wasps, the selective consequences of any particular brood sex ratio are immediately realized. By contrast, adult life-spans in most vertebrates are relatively long, resulting in complications that arise from overlapping generations (10) or interactions between siblings (6).

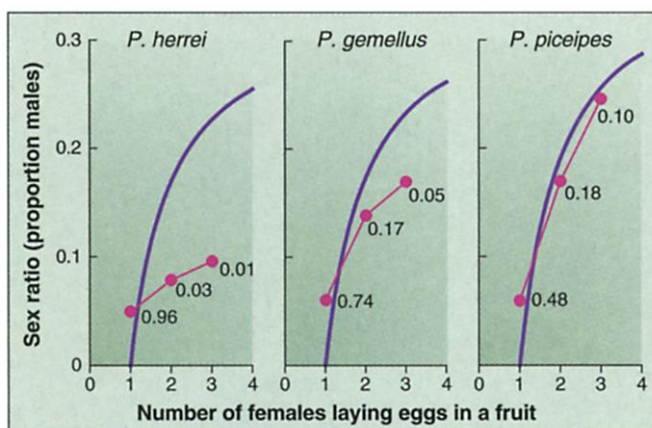
Using Sex Allocation to Infer Characteristics of a Population

Sex allocation provides an easy way to estimate population characteristics that are technically difficult or expensive to measure

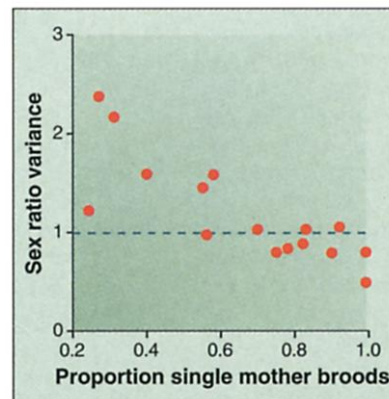
directly (11). One example, with potentially important benefits, is the use of sex allocation patterns in malaria (*Plasmodium*) and other protozoan parasites to infer the amount of inbreeding (also called the selfing rate, which is defined as the proportion of a female's daughters that are fertilized by her sons). Because the rate of inbreeding in these parasitic species can influence the evolution of resistance to vaccines and drugs, inbreeding estimates are important for designing effective control and treatment programs (12).

Direct measures of the inbreeding rate using molecular genetics can be difficult to obtain, and past estimates have been extremely controversial (12). Sex allocation theory allows the inbreeding rate to be estimated more readily because the occurrence of inbreeding skews the sex ratio of offspring in favor of females, analogous to the situation in fig wasps (13, 14). The higher the level of inbreeding, the greater is the predicted female bias of the sex ratio. The amount of inbreeding (F) can be predicted from the observed sex ratio (r) by the refreshingly simple equation $F = 1 - 2r$. Consequently, if we assume that the sex ratio theory is correct, then the inbreeding rate can be estimated from sex ratio data. An advantage of this method is that sex ratio data can be collected relatively easily from a number of populations and species, allowing generalizations to be made (see the figure, next page). In cases where both indirect sex ratio data and direct genetic estimates of the inbreeding rate are available, they are in quantitative agreement, supporting the use of this approach (14).

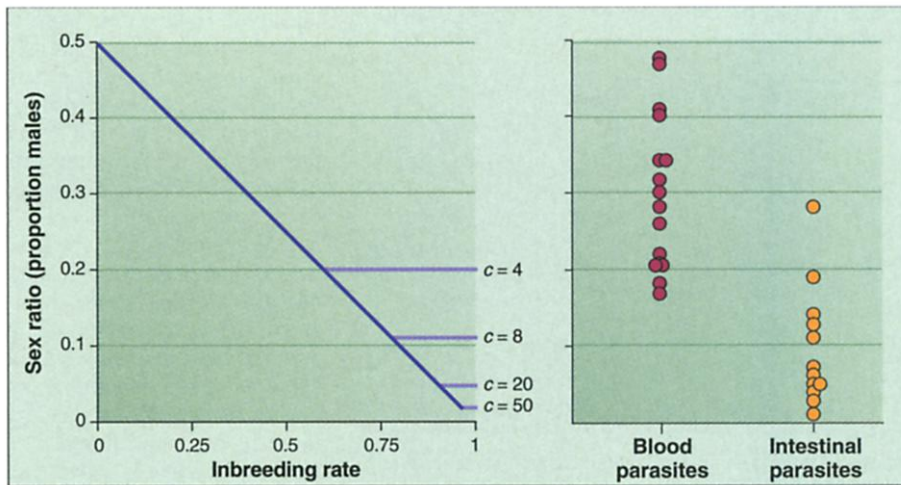
More generally, sex allocation theory applied to protozoan parasites provides support for the application of evolutionary optimization models to infectious disease research (14). Despite having to assume equilibrium states (not an obvious feature of microparasite populations), simple theory is able to explain variation in a life-



A fig of one's own. Precision of adaptation and the sex ratios of fig-pollinating wasps. Shown are the observed sex ratios (circles) and theoretical optima (curved lines) for different numbers of female fig-pollinating wasps (from three species of the genus *Pegoscopus*) laying eggs in a fruit. The numbers next to the circles show the relative frequency with which that number of females lays eggs in a fruit in nature—that is, in the fig species pollinated by *P. herrei*, 0.96 of fruit have only one female enter (pollinate and lay eggs), 0.03 have two females enter, and 0.01 have three enter. The observed sex ratios of progeny are closest to theoretical situations (number of females laying eggs in a fruit) that are encountered most frequently (4). The observed shifts in sex ratio are greatest in species where the number of females laying eggs in a fruit is more variable.



Broody female wasps. The variation (observed/binomial) in sex ratios of offspring when only one female lays eggs in a fig fruit (the dashed line represents binomial variance). The more frequently only one female lays eggs in a fig fruit, the less variation there is in the sex ratio of the progeny. [Adapted from (5)]



More daughters for inbreeders. (Left) Sex ratios of progeny can be used to estimate the rate of inbreeding in protozoan blood parasites such as those causing malaria. The predicted sex ratio (proportion of gametocytes that are male) is plotted against the inbreeding rate (14). When the rate of inbreeding is high, the sex ratio is constrained by the need to produce enough male gametes to fertilize female gametes. This constraint is determined by c , the mean number of viable gametes released by a male gametocyte (c is equivalent to the maximum number of times that a male could mate). (Right) Sex ratios can be used to estimate the rate of inbreeding in the malaria parasite *Plasmodium* and in the intestinal parasite *Toxoplasma*. Sex ratios of progeny in malaria (and other blood parasites) are extremely variable, suggesting that the inbreeding rate also varies enormously. This is not unexpected given that the degree of inbreeding is likely to depend on infection rates: The greater the number of parasites infecting a host, the lower will be the rate of inbreeding (14). In contrast, the greater female bias in the sex ratios of the progeny of intestinal parasites such as *Toxoplasma* suggests higher rates of inbreeding.

history trait (sex ratio) across a taxonomically diverse range of protozoan parasites (14, 15). In particular, it provides a clear demonstration of the importance of population structure in determining natural selection in parasitic protozoans. Theory suggests that the virulence of parasites should respond to the same changes in population structure (16).

Applying sex allocation to the prediction of characteristics of a population or species has great potential because inferences can be made about any factor that influences the optimal pattern of sex allocation (11). For example, it can be very difficult to determine whether animals recognize kin and, if they do, the cues that are involved. In social insects—ants, bees, and wasps—workers adjust the sex ratios of offspring in response to their relatedness to the males and females that they are raising, indicating that workers have a mechanism for accurately recognizing kin (17). To what extent nonsocial insects recognize kin, and whether kin recognition has facilitated the evolution of social behavior is less well established. Local mate competition (LMC) theory allows us to test for kin recognition in solitary wasps and bees: Discriminating females should produce a more female-biased sex ratio if they mate with a sibling rather than outbreeding with unrelated males (3, 18).

Initially driven by attempts to explain the sex ratios of seemingly obscure insect species, sex allocation studies are now yielding valuable evidence in support of the theory of evolution by natural selection, and are also proving important for elucidating the biology of protozoan parasites.

References and Notes

- In large populations, each individual of the rarer sex would make a greater genetic contribution to the next generation (frequency-dependent selection). Hence, equal numbers of males and females is the evolutionarily stable strategy. However, in many situations a bias in sex allocation is favored in ways that are clearly predicted by theory (for example, under certain circumstances some individuals in a population will do better by producing sons, and others by producing daughters; in other circumstances, all individuals may do better by producing more of one sex) [E. L. Charnov, *The Theory of Sex Allocation* (Princeton Univ. Press, Princeton, NJ, 1982); H. C. J. Godfray and J. H. Werren, *Trends Ecol. Evol.* **11**, 59 (1996); S. A. Frank, *Foundations of Social Evolution* (Princeton Univ. Press, Princeton, NJ, 1998); D. R. Campbell, *Trends Ecol. Evol.* **15**, 227 (2000)].
- Studies of sex allocation have provided some of the best quantitative evidence for the relative importance of selection at the gene, individual, kin, and population levels [J. Seger and J. W. Stubblefield, in *Adaptation*, M. R. Rose and G. V. Lauder, Eds. (Academic Press, San Diego, CA, 1996), pp. 93–123; W. D. Hamilton, *Narrow Roads of Gene Land I, Evolution of Social Behaviour* (Freeman, Oxford, 1996); M. Chapuisat and L. Keller, *Heredity* **82**, 473 (1999)].
- W. D. Hamilton, in *Reproductive Competition and Sexual Selection in Insects*, M. S. Blum and N. A. Blum, Eds. (Academic Press, New York, 1979), pp. 167–220; E. A. Herre, *Science* **228**, 896 (1985); S. A. Frank, *Evolution* **39**, 949 (1985).
- E. A. Herre, *Nature* **329**, 627 (1987).
- S. A. West and E. A. Herre, *Evolution* **52**, 475 (1998).
- The observed variations in sex ratios are predicted by LMC theory. If n females each lay equal numbers of offspring in a fruit, the predicted sex ratio is $(n-1)/(2-s)/n(4-s)$, where s is the proportion of females that mated with a brother (3). Explanations based on selection at the individual and kin level suggest that the female-biased sex ratio is favored because it reduces competition between brothers. If only one female lays eggs in a fruit, then the extent of LMC is at its greatest (all males competing for mates are brothers), and so she is predicted to produce a sex ratio of 0, which is interpreted to mean producing just enough sons to mate with her daughters. As the number of females laying eggs in a fruit increases, the extent of competition for mates between brothers (LMC) is reduced, and so a less female-biased sex ratio is favored [W. D. Hamilton, *Science* **156**, 477 (1967); P. D. Taylor, *Nature* **291**, 64 (1981)].
- N. H. Barton and M. Turelli, *Annu. Rev. Genet.* **23**, 337 (1989).
- J. Komdeur *et al.*, *Nature* **385**, 522 (1997); L. E. B. Kruuk *et al.*, *Nature* **399**, 459 (1999); B. C. Sheldon *et al.*, *Nature* **402**, 874 (1999).
- Variation in environmental predictability can also explain patterns among different parasitic wasp species. In many species of parasitic wasp, where only one individual can develop per host, females lay male eggs on small hosts and female eggs on large hosts. This is presumed to be advantageous because females gain a greater benefit from the resulting increase in body size. Females are less likely to adjust the sex of their offspring in response to host size when hosts are not killed and continue to grow after she has laid her egg. In this case the size of a host is a less reliable cue of resources available for offspring development [B. H. King, *Oecologia* **78**, 420 (1989)].
- J. H. Werren and P. D. Taylor, *Am. Nat.* **124**, 143 (1984); S. A. West and H. C. J. Godfray, *J. Theor. Biol.* **186**, 213 (1997).
- The most useful areas for using sex allocation in this way will be those where there is sufficient knowledge of relevant biology to make correct assumptions, and where extreme sex ratio shifts can be expected.
- M. Tibayrenc, *Adv. Parasitol.* **36**, 47 (1995); R. E. L. Paul and K. P. Day, *Parasitol. Today* **14**, 197 (1998).
- In several groups of protozoan parasites the life-cycle has a sexual stage (the gametocyte or gamont). LMC can favor female-biased sex ratios when mating takes place, not at random within the population, but among a small fraction of the population (14). For example, in blood parasites such as malaria (*Plasmodium*), gametocytes in a single blood meal (imbibed by a mosquito) usually mate, and so mating only takes place between the same parasite genotype. In contrast, in the intestinal parasite *Toxoplasma*, mating usually takes place between different parasite genotypes infecting the same local area of the host gut. LMC theory applied to protozoan parasites suggests that the predicted sex ratio (r) can be related to the inbreeding rate (F) by the equation $r = (1-F)/2$. (F , the inbreeding coefficient, is the probability that two homologous genes in two mating gametes are identical by descent.) The sex ratio is predicted to decline from 0.5 for complete outcrossing ($F=0$) to 0 for complete inbreeding ($F=1$).
- A. F. Read *et al.*, *Proc. R. Soc. London Ser. B* **260**, 359 (1995); J. Pickering *et al.*, *Evol. Ecol. Res.* **2**, 171 (2000); S. A. West, T. G. Smith, A. F. Read, *Proc. R. Soc. London Ser. B* **267**, 257 (2000).
- D. Shutler and A. F. Read, *Oikos* **82**, 417 (1998); R. E. L. Paul *et al.*, *Science* **287**, 128 (2000); S. Reese and A. F. Read, *Trends Ecol. Evol.* **15**, 169 (2000).
- S. A. Frank, *Q. Rev. Biol.* **71**, 37 (1996).
- J. J. Boomsma and A. Grafen, *Evolution* **44**, 1026 (1990); U. G. Mueller, *Science* **254**, 442 (1991); L. Sundstrom, *Nature* **367**, 266 (1994); J. D. Evans, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6514 (1995); L. Sundstrom, M. Chapuisat, L. Keller, *Science* **274**, 993 (1996); L. Sundstrom and J. J. Boomsma, *Proc. R. Soc. London Ser. B* **267**, 1439 (2000).
- J. M. Greeff, *J. Evol. Biol.* **9**, 855 (1996).
- We thank P. Awadalla, A. Griffin, L. Kruuk, A. Read, S. Reese, T. Solomon, and R. West for useful comments.