tool in the study of Na^+,K^+-ATPase structure and mechanism. The understanding of cardiac glycoside/enzyme interaction also has practical significance because of the therapeutic use of this class of compounds in the treatment of congestive heart failure.

The ability to assess the biological activity of the Na^+,K^+-ATPase α_1 subunit gene via direct DNA transfer raises two additional points of interest. The construction of chimeric α subunit cDNAs, coupled with the application of site-directed mutagenesis, should allow the identification of other functional domains within the Na^+,K^+-ATPase. This approach should also prove useful for defining the functional differences between α subunit isoforms. Finally, our results demonstrate that the full-length mouse α_1 subunit cDNA can be used as a dominant selectable marker for somatic cell genetic studies utilizing primate and other ouabain-sensitive cells.

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Short Interval Time Measurement by a Parasitoid Wasp

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The number of eggs laid by the parasitoid wasp *Trichogramma* varies with host volume. The duration of the wasp's initial transit across the host surface during host examination is used to determine the number of eggs laid. A 2.5-second reduction in initial transit resulted in a 30% reduction in eggs oviposited, demonstrating that these wasps measure short time intervals. This measure is used for progeny allocation independent of host body size.

EACH YEAR A GREATER NUMBER OF *Trichogramma* (*1*) wasps are reared for biological control applications than any other animal used by man (*2*). These parasitoids have been used in massive release programs against a variety of pest insects, including the cotton bollworm (*Heliothis zea* Boddie) (*3*), the European corn borer (*Ostrinia nubilalis* Hb.) (*4*), and the sugarcane borer (*Diatraea saccharalis* Guilding) (*2, 4*). Although minute (adult length, 0.3 to 0.8 mm), *Trichogramma* display a range of complex behaviors. Since 1982 we have investigated the behavior and sensory physiology of *Trichogramma minutum* Riley to determine methods of improving their efficacy in biological control. We have also used *Trichogramma* as a vehicle for understanding the mechanisms that underlie the processing of mechanosensory information in the nervous system.

Trichogramma develop as parasitoids within the eggs of various other insects. The host eggs differ considerably in size, ranging from 0.3 mm to more than 2.5 mm in diameter. The size of the host determines the number of eggs (clutch size) laid by *Trichogramma* (*5–7*). Fewer eggs are allocated to smaller hosts, thereby minimizing larval competition and mortality resulting from limited nutrients and space (*8*). However, if too few eggs are laid into each host, the wasp may be unable to find sufficient hosts to dispose of its egg complement during its 2- to 6-day adult life span. In addition, sufficient larvae must develop within the host to consume most of the contents, as excess host residues can increase larval mortality (*9*). As a result, *Trichogramma* adjust the number of progeny to host volume, increasing clutch size proportionally with host size (*6, 7*).

Until recently, the mechanism by which *Trichogramma* measure host volume was unknown. In an earlier study (*7*), we found that the response of *Trichogramma* to host volume depends on mechanosensory rather than visual or chemical cues. The wasps allocate fewer progeny to *Manduca sexta* (L.) (*10*) hosts partially embedded in the substrate than to fully exposed hosts of the same species, both in daylight and in complete darkness. Since both embedded and fully exposed hosts have the same diameter, internal content, surface odor, and texture, the wasps cannot use these parameters to discriminate between hosts that differ only in exposed surface area (*7*).

To assess the use of external cues by the wasp, we performed a detailed analysis of its behavior on the host surface. Before oviposition, the wasp examines the host surface by walking over it, simultaneously drumming the surface with its antennae. When it encounters the juncture between the host and substrate, it makes an abrupt turn and continues its examination of the host surface (*5–7*). During its walk, the wasp may make many such contacts and turns while remaining on the host surface. Several of the parameters of this examination walk, including the number and frequency of substrate contacts and the intervals between them, depend on the exposed surface area of the host. Total time spent examining the host surface, however, is set by the wasp in response to host curvature, not to exposed surface area (*7*).

To determine which parameter was used by the wasps to set clutch size, we observed individual *Trichogramma* (*11*) during host examination on spherical *M. sexta* eggs (*12*) and recorded the frequency and intervals between substrate contacts and turns (*13*). These data were subsequently compared with the number of progeny allocated to the host (*14, 15*). Seven parameters were considered: total number of substrate contacts, mean interval between contacts, interval between last contact and oviposition, longest and shortest interval between contacts, total interval between first three contacts, and the interval between the first contact with the host and the first substrate contact (initial transit duration). Of these, only the initial transit duration showed a significant linear relation with progeny allocation [slope,

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Table 1. Effect of wasp size on the number of final instar larvae per host (*12, 14, 18*) (means ± SD).

Treatment group	*n*	Wasp head length (mm)	Host diameter (mm)	Progeny per host	Volume of host per larva (mm^3)
Small wasps	14	0.174 ± 0.011	1.56 ± 0.06	18.8 ± 8.1	0.106
Large wasps	22	0.255 ± 0.007	1.57 ± 0.06	21.5 ± 5.6	0.94

1.14 ± 0.18 (SE); $n = 33$, $P < 0.01$]. As the duration of the wasp's initial transit across the host surface increases, more eggs are laid. The path taken during this initial transit generally follows a straight great circle route across the host surface, bringing the wasp close to the highest point of the host. If the wasp maintains a constant walking speed, the duration of the initial transit could give information about the exposed area of the host.

To confirm the role of initial transit in setting progeny allocation, we placed a small plastic shield over the *Manduca* host in the path of the wasp. As soon as the wasp contacted the shield and turned, the shield was removed. In this way, the duration of the initial transit was reduced to 2.9 ± 1.2 seconds ($n = 26$) compared to 5.4 ± 1.5 seconds ($n = 24$) for wasps whose transit was uninterrupted ($P < 0.001$) (*13, 16*). After removal of the shield, the wasps were allowed to continue their examination walk over the entire exposed host surface. These wasps allocated an average of 19.1 ± 6.9 progeny per host, significantly fewer than the 30.0 ± 5.6 progeny per host allocated by the wasps that made complete initial transits ($P < 0.001$) (*14*).

These results demonstrate that parameters dependent on the initial transit are used by the wasp to measure exposed host volume. The use of the initial transit raises two questions. First, what cues about the dimensions of the initial transit are used? Second, does the response of the wasp depend on its own size, relative to that of the host? The latter question is of particular concern since the size of the individual adult wasp is determined phenotypically, depending on the quantity of nutrient available to the larva (*8, 17*). If relative measures are used by *Trichogramma*, then smaller wasps would be expected to lay more eggs into a host than larger wasps, resulting in smaller progeny of reduced fitness (*8*). As a result, a phenotypic trait of lower fitness could be transmitted between generations. However, our experiments demonstrate that this effect does not occur.

When we presented large and small wasps (*18*) with hosts of constant size (*19*), the number of progeny allocated per host (*11*) remained essentially constant ($P > 0.10$) (Table 1). Therefore, the wasps must use a measure of the initial transit that is independent of their body size. Direct measures of the distance walked cannot be used, as these would depend on the dimensions of the wasp. For instance, the total number of steps taken is determined by stride length, which is proportional to body length (*20*). However, the duration of the initial transit is virtually the same for both large and small wasps walking on hosts of constant diameter and exposed volume ($P > 0.10$) (Table 2) (*13, 21*). The constancy of initial transit duration also shows that both large and small wasps walk at the same speed during host examination. Thus, time is a parameter whereby the wasps can measure absolute host volume.

The ability to measure absolute rather than relative volumes ensures that larvae of all sizes of wasps are provided a constant optimal volume of host (Table 1); therefore, larvae are subject to similar levels of competition for nutrients. Thus, the progeny of *Trichogramma* are not disadvantaged by the phenotypic size of their parent. To respond appropriately to host volume, the wasp need only measure the time elapsed between its initial contact with the host and its first subsequent contact with the juncture between the host and substrate. Since this mechanism requires only mechanosensory information and an internal timer, it should also function in darkness. Such an ability to discriminate between hosts in darkness has been shown (*7*).

The results of the interrupted initial transit experiment indicate that the wasps are sensitive to relatively small periods of time. A 2.5-second difference in transit duration results in a difference of 11 progeny per host (30%). Since the wasps are able to measure hosts as small as 0.1 by 0.3 by 0.3 mm [for example, *Sitotroga cerealella* (Olivier) (*22*)], which are allocated only a single egg each, they may be able to measure durations ranging from less than 0.5 second to more than 8 seconds.

In addition to demonstrating that *Trichogramma* can respond to relatively small differences in time, the results show that these animals can use time intervals to obtain specific, quantitative information about the geometric or topographic configuration of their local environment. In this process a temporal pattern of events is interpreted to provide spatial information about the surroundings of the wasps. Similar use of time cues may be involved in the behavior of other animals, including the complex orientation and nest-building activities of ants, wasps, and bees, in which spatial information could also be encoded in a temporal sequence of actions.

For *Trichogramma*, the ability to measure time has permitted the exploitation of a wide range of host species and sizes. By coupling a relatively simple measure to clutch size, *Trichogramma* have acquired the necessary flexibility to deal with complex problems of resource assessment despite the limitations of their small size and miniature sensory and central nervous systems.

Table 2. Effect of wasp size on examination behavior (*12, 13, 18, 21*) (means ± SD).

Treatment group	*n*	Wasp head length (mm)	Host diameter (mm)	Duration of initial transit (seconds)
Small wasps	27	0.172 ± 0.017	1.37 ± 0.17	5.7 ± 3.5
Large wasps	28	0.243 ± 0.015	1.36 ± 0.15	5.7 ± 3.4

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11. In all experiments, *T. minutum* Riley reared on *M. sexta* were used. Cultures were maintained for 65 generations in the laboratory at 24° to 27°C, and 30 to 40% relative humidity. Female parasitoid wasps used were 18 to 24 hours old, mated, and unfed. Each wasp was used only once.
12. Eggs of *M. sexta* were used as hosts for all experiments. They were obtained from Carolina Biological Supply Company and killed by freezing (−12°C). These eggs were used 5 to 6 days after oviposition and measured individually by using an ocular micrometer. There was no significant difference in mean host diameter between treatment groups within experiments ($P > 0.25$).
13. Times of host examination events were recorded during direct observation in real time by using a computer-based event recorder. The Microsoft BASIC program was run on a TRS-Model 100 portable computer.
14. Progeny per host was determined as the number of final instar larvae per host 5 days after oviposition. Counts were obtained by dissection of the hosts.

15. Hosts were mounted in 1.34- and 1.00-mm diameter holes in white plastic squares (2 by 2 cm). Each wasp was allowed to complete examination and oviposition. The wasps were observed individually to prevent repeated parasitization of the same host. Trials in which the wasp left the host before completing oviposition were rejected.
16. Mean ± SD was used throughout. Statistical significance was determined by t tests.
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18. Head length was measured from the medial ocellus to the tip of the closed mandibles by using an ocular micrometer. Wasps differed significantly in mean head length between large and small treatment groups ($P < 0.001$).
19. Single hosts were mounted on white cardboard squares (2 by 2 cm) with gum arabic. After host examination was completed, wasps were observed as in (*15*).
20. Measurements made from films of the initial transit demonstrate a significant linear relation between wasp body length and stride length [slope, 0.58 ± 0.064 (SE); $n = 15$, $P < 0.01$].
21. Wasps were observed on single hosts mounted on cardboard cards with gum arabic. Only wasps that completed their host examination and began ovipositing were included in the data. For details of methods and results, see J. M. Schmidt and J. J. B. Smith [*J. Exp. Biol.* **129**, 151 (1987)].
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The Three-Dimensional Structure of Asn^{102} Mutant of Trypsin: Role of Asp^{102} in Serine Protease Catalysis

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The structure of the Asn^{102} mutant of trypsin was determined in order to distinguish whether the reduced activity of the mutant at neutral *p*H results from an altered active site conformation or from an inability to stabilize a positive charge on the active site histidine. The active site structure of the Asn^{102} mutant of trypsin is identical to the native enzyme with respect to the specificity pocket, the oxyanion hole, and the orientation of the nucleophilic serine. The observed decrease in rate results from the loss of nucleophilicity of the active site serine. This decreased nucleophilicity may result from stabilization of a His^{57} tautomer that is unable to accept the serine hydroxyl proton.

THROUGHOUT THE DIVERSE FAMILY of serine proteases, the three residues implicated in the bond breaking and making events of protease catalysis, His^{57}, Asp^{102}, and Ser^{195} (chymotrypsin numbering system) are conserved. The spatial relation among these residues is virtually equivalent in the three-dimensional structures of all serine proteases studied. The catalytic roles of Ser^{195} and His^{57} are firmly established (*1*). The substrate (ester or amide) carbonyl carbon undergoes a nucleophilic attack by the hydroxyl group of Ser^{195}, which leads to the formation of an acyl enzyme intermediate. His^{57} functions as a catalytic base by assisting in the transfer of a proton from the serine hydroxyl to the substrate leaving group. The role of Asp^{102} has not yet been defined. The three functions proposed for this residue are: (i) stabilizing the His^{57} conformation that is required for catalysis (*2*), (ii) stabilizing the appropriate His^{57} tautomer (*2*), and (iii) stabilizing the positively charged histidine that forms during the reaction (*3*). The proposed functions were tested with a genetically engineered mutant of the anionic isozyme of rat trypsin that was constructed by replacing Asp^{102} with an asparagine (*4*), designated here as D 102 N trypsin, where D is Asp and N is Asn.

The activity of D 102 N trypsin has been studied as a function of *p*H (*4*). The activity of this mutant enzyme toward a variety of substrates is reduced by four orders of magnitude relative to trypsin between *p*H 7 and *p*H 9, where the latter is optimally active. The Michaelis constant, K_m, of the mutant enzyme is virtually unaffected (*4*). This raises the question of whether the chemical properties of the asparagine itself or the conformational differences in the enzyme are responsible for the loss of activity in D 102 N trypsin. To address this point, we describe the three-dimensional structure of D 102 N trypsin at both *p*H 6 and *p*H 8.

Orthorhombic crystals (space group $P2_12_12_1$) of rat D 102 N trypsin grown at *p*H 6 in the presence of benzamidine were

Fig. 1. An α-carbon diagram (stereoscopic) of anionic rat D 102 N trypsin at *p*H 6 (*9–12*) (green) is superimposed on bovine trypsin (blue). Residues in rat trypsin (*12*) that differ in side-chain type from corresponding residues in the bovine sequence (*25*) are highlighted in red here. Side-chain positions for residues Asn^{102}, His^{57}, and Ser^{195} are also shown in red. The root-mean-square (rms) difference in position between corresponding atoms of D 102 N rat trypsin in the crystals grown at *p*H 6 and bovine trypsin (*13, 26*) after least-squares superposition is 0.47 Å for all main-chain atoms and 0.67 Å for all side-chain atoms. Values quoted are the average of those obtained for molecules 1 and 2 in the asymmetric unit of the D 102 N trypsin crystals grown at *p*H 6. The computed rms distance may be an underestimate of the true differences in the two structures because of the use of bovine trypsin as the initial phasing model. The rms difference after superposition between all atoms of the two molecules in the asymmetric unit is 0.21 Å. The rms deviation between the main-chain atoms of the *p*H 6 and *p*H 8 crystal forms of D 102 N trypsin is 0.25 Å.

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