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Behavioral genetics of thermosensation and hygrosensation in *Drosophila*

(temperature preference/humidity preference/antenna/arista/neurogenetics)

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ABSTRACT **Whereas temperature and humidity are critical variables affecting physiology, behavior, and evolution, the genetic and neuronal underpinnings of thermosensation and hygrosensation remain poorly understood. We have initiated a behavioral-genetic investigation of these sensory systems in *Drosophila*. Behavioral tests are described for the rapid screening of mutants defective in thermosensation and hygrosensation. We demonstrate the strong responses of normal flies to temperature and humidity. Two mutants were found with defects in thermosensation, only one of which is also defective in hygrosensation, indicating that they involve different sensory mechanisms. Ablation experiments further separate these sensory systems by showing that thermoreceptors are housed in the third antennal segment, whereas hygroreceptors are located more distally in the antennal arista.**

Environmental temperature and humidity are major determinants of the geographic distribution of terrestrial fauna (1, 2). Poikilothermic species are constrained by the temperature conditions in which they can function and thus have limited geographic ranges. Given that temperature varies both spatially and temporally during each day, poikilotherms must actively track a suitable thermal regime, requiring constant sensory monitoring of the environment. This is particularly crucial for small-bodied poikilotherms; on entering direct sunlight, a 10-mg fly can heat up by 10°C in only 10 sec (3)! For *Drosophila melanogaster*, which weighs in at only 1 mg, this is an even more severe concern. On the other hand, homeothermic species, due to their physiological thermoregulatory mechanisms (4), are more tolerant to varied temperatures and therefore may occupy diverse habitats. Yet, for homeotherms too, behavior plays an important role in thermoregulation (5, 6). Behavioral thermoregulatory responses have been recorded in a wide variety of animals by examining their temperature preference on a thermal gradient. A few examples are bats (7), lizards (8), turtles (9), fish (10), worms (11), ants (12), beetles (13), cockroaches (14), and flies (15). However, the mechanisms mediating thermosensation remain poorly described.

Behavioral thermoregulation is also a conspicuous aspect of human behavior, as noted by seasonal variations in attire, travel, and lifestyle. Experimental studies have revealed large individual differences in preferred temperature (for review, see ref. 16). In one such study, 25°C was preferred by the majority of subjects, but a fifth of subjects preferred less than 20°C and an equal number preferred more than 30°C (16). It is indeed common, within a family, to suffer domestic disharmony over the setting of the thermostat; preferences may be markedly different, not only between parents, but also among their sibling children. Nevertheless, the possible role of genes in determining temperature preference remains unexplored. This can be approached using *Drosophila* as a model system.

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Along with temperature, relative humidity is important due to its impact on the opposing requirements for evaporative cooling and body water maintenance. Preferences for different relative humidities have been demonstrated in various animals including birds (17), lizards and toads (18), beetles (19), and flies (20), but, again, the mechanisms for hygrosensation and their distinction from those of temperature sensation remain elusive. Mutations separately affecting one or the other can offer an incisive approach.

In vertebrates, though the morphologies of various thermoreceptors have been characterized (21–23), the central pathways remain poorly defined. Furthermore, the mechanisms by which thermal stimuli depolarize the sensory nerve endings to trigger action potentials remain unknown. In cases such as the rattlesnake's thermoreceptive pit organ, the sensitivity defies the imagination; a temperature change of 0.003°C can trigger a physiological response (24)! In insects, electrophysiological responses to thermal and hygro stimuli have been recorded from the antennae of several species (refs. 25 and 26; for reviews, see refs. 27–30), but the neurons and mechanisms involved remain to be identified.

The present state of knowledge concerning the underpinnings of thermo- and hygrosensation is comparable to what was known two decades ago about visual transduction, circadian rhythm, chemosensation, and learning and memory. The phenomena were well-established, but understanding of the mechanisms left much to be desired. The application of neurogenetic analysis to behavior, through single-gene mutants in *Drosophila*, has played a major role in revealing the neural, molecular, and genetic bases of these modalities (for reviews, see refs. 31 and 32). The first step in each case was the development of a simple behavioral paradigm suitable for the rapid screening of mutants. In this article, we describe behavioral tests for responses of *Drosophila* to temperature and humidity, and show that these responses can be changed by single-gene mutations as well as by surgical manipulations. Mutations and selective ablations affecting thermal preference have also been demonstrated in the nematode, *Caenorhabditis elegans*, and neurons critical for thermotaxis have been identified (11, 33, 34). This provides the exciting prospect of a comparative neurogenetic approach to thermosensation in these two organisms.

MATERIALS AND METHODS

Fly Strains and Culture Conditions. *D. melanogaster* of the Canton-Special (C-S) wild-type strain and many extant mutant strains were examined. The behavior of the wild-type strain and the defective behavior of two of these mutants are described here. The first was originally isolated in our laboratory in a countercurrent phototaxis screen and designated SB8 (35); we have now renamed it *bizarre*. The mutation is located between 12B6–7 and 15F1–3 on the X chromosome; in

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recombination mapping, it acts as a single locus (D. Kretzschmar, personal communication). The second mutant is the third-chromosomal homeotic mutant *spineless*^{aristapedia} (36), obtained from the Umeå *Drosophila* Stock Center (Sweden). All flies were raised at 25°C on a standard cornmeal medium in foam-stoppered half-pint milk bottles. Whereas the relative humidity of the culture room was 60%, inside the bottles it was close to saturation. Humidity measurements were made with a digital hygrometer probe [Omega Engineering (Stanford, CT) model RH-30–3]. For all tests, approximately equal numbers of males and females were used for each genotype. No sex- or age-related differences in the behaviors under study were apparent. Flies were handled with a mouth aspirator and were never subjected to anesthesia before testing.

Temperature Preference Tests. To study thermosensation, the temperature preference of *Drosophila* was examined on a linear thermal gradient (Fig. 1*a*). The gradient was produced by resting an aluminum slab (27 × 18 × 2.5 cm) on a cold plate [Teca Corp. (Chicago) model LHP-800CP] under one end and a hot plate (Corning model PC320) under the other, with thermal joint compound [Thermalloy (Dallas) no. 249] applied to the interfaces. To keep the relative humidity uniform along the gradient, the aluminum slab was covered with a moist sheet of chromatography paper (Whatman no. 3030 917) that was marked into 10 observation fields (Fig. 1*a*, dashed lines in top view) for recording the distribution of flies. Temperature along the gradient, measured on the paper with a thermocouple, was stable, reproducible, and linear, with a slope of 0.6°C per cm. For different experiments, the range was set at either 18–31.5°C or 23–36.5°C. A 0.5-cm high Plexiglas cover confined the flies to the gradient. The cover included three plastic strips (Fig. 1*a*, horizontal lines in top view) providing channels

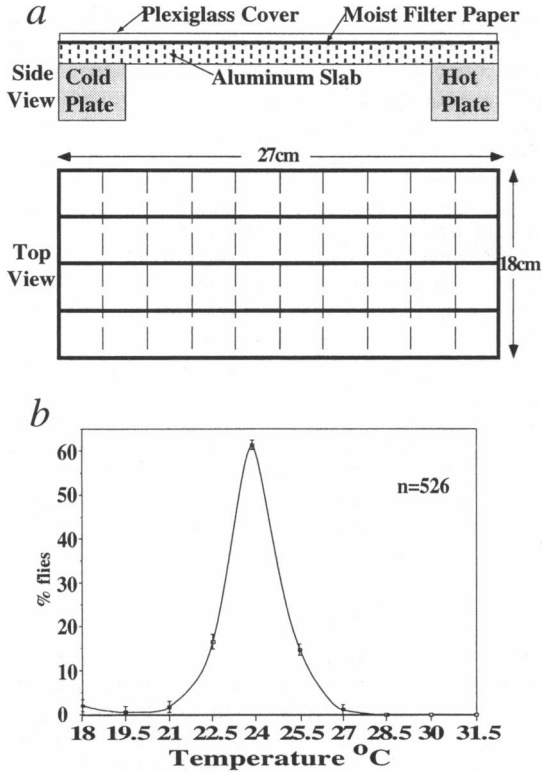

FIG. 1. Temperature gradient paradigm. (*a*) Linear thermal gradient for temperature preference tests. See text for details. (*b*) Temperature preference responses of wild-type *Drosophila* tested on a 18–31.5°C gradient. Flies exhibited a strong preference, peaking at ≈24°C. Data pooled from 526 flies, about 10 flies per test. Error bars represent standard errors of the mean.

to test four groups simultaneously. To prevent flies from escaping the temperature gradient by resting on the walls or roof of the cover, the latter was lightly coated with quinine sulfate powder, an aversive stimulus for *Drosophila* (37). Tests were conducted in darkness.

Temperature Choice Tests. An alternative, binary test for thermosensation involved giving flies a choice between 22°C (room temperature) and 30°C in a Plexiglas choice-chamber apparatus (Fig. 2*a*), earlier used in learning experiments (38). Each choice tube (polystyrene, Falcon no. 2017) was fitted into an aluminum ring; around one of these rings was wrapped a band heater [McMaster-Carr (Los Angeles) model 3594K72] powered by a temperature controller (Fuji model PYZ4) so that the inside temperature was 30°C. The tubes were rinsed in 95% ethanol and air-dried before use. Tests were conducted under diffuse red illumination (Kodak safelight, model C).

Humidity Choice Tests. To study hygrosensation, flies were given a choice between moist and dry air in a choice-chamber apparatus modified for air flow (see Fig. 6*a*). Purified, dehydrated compressed air [Air Liquide (Santa Fe Springs, CA) Ultra Zero Grade] was used; dry air was taken directly from the source, whereas moist air was produced by bubbling through deionized H_2O. Air was delivered to each side of the chamber through holes drilled at the ends of the choice tubes (polystyrene, Falcon no. 2017). Both air streams were at room temperature as measured with a thermocouple. Air flow in each arm of the choice-chamber was set at 500 ml/min and monitored with a microflowmeter [Gilmont (Great Neck, NY) no. 12]. The relative humidity, measured with the digital hygrometer, was 3% in one arm and 99% in the other. Spaces along the sides of the elevator served as air exit paths, thus producing sharp humidity steps at the edges of the central choice point. All tubes were rinsed in 95% ethanol and air dried before use. Tests were conducted under diffuse red illumination (Kodak safelight, model C).

Ablation Techniques. For ablation, a fly was held in a modified pipette tip, with the head protruding, providing access to the antennae without anesthesia (39). The third antennal segments were removed using fine forceps. Aristal ablations were done with a pulsed ruby laser aimed at the

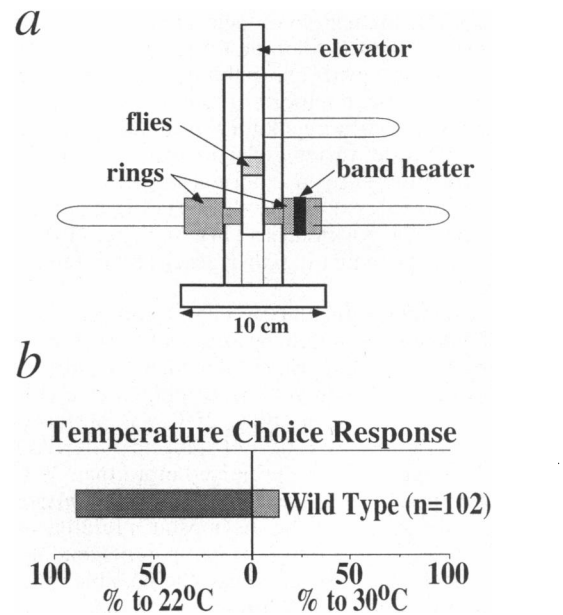

FIG. 2. Temperature choice paradigm. (*a*) Binary choice-chamber apparatus for testing thermosensation. See text for details. (*b*) Temperature choice responses of wild-type *Drosophila*. When given a choice between 22°C and 30°C, flies strongly chose the 22°C arm of the chamber. Data pooled from 102 flies, about 10 flies per test.

insertion point of the arista into the third antennal segment. At least 2 hr intervened between surgery and testing. The behavior of surgically manipulated flies remained the same even if this interval was extended to several days.

RESULTS

Responses to Temperature. *Temperature gradient paradigm.* To investigate thermosensation, the temperature preference of *Drosophila* was examined on a linear thermal gradient ranging from 18°C to 31.5°C (Fig. 1). Given the interdependence of temperature and relative humidity, and its confounding effect on investigations of temperature preference, discussed by Andrewartha and Birsch (2), we tested temperature preference under the uniformly high relative humidity produced by covering the thermal gradient with a moist sheet of filter paper (Fig. 1*a*). For testing, approximately 10 flies were transferred from a culture bottle to each channel of the gradient through holes in the cover. Flies were allowed 20 min to distribute and then anesthetized by introducing CO_2; the number of flies per observation field was recorded. The responses of multiple batches of 10 flies were pooled.

Wild-type flies showed a strong temperature preference, peaking at ≈24°C (Fig. 1*b*). This preference was unaffected by prior acclimatization for 5 days to either 18°C or 29°C (data not shown). This is in contrast with *C. elegans*, in which temperature preference is quite plastic; when the maintenance temperature is changed before testing, the preference of the worms shifts to that temperature within a few hours (11). Parallel genetic analysis of these two organisms is therefore likely to be especially interesting in illuminating similarities and differences in basic mechanisms.

Temperature choice paradigm. A second test for thermosensation, designed for the rapid screening of mutants, involved giving flies a choice between 22°C and 30°C in a choice-chamber apparatus (Fig. 2*a*). For each test, approximately 10 flies were removed from a culture bottle and placed in the upper tube shown in the Fig. 2*a*. They were then transferred to the elevator by tapping, and the elevator was moved down to the halfway position shown in the Fig. The flies were brought to the choice point by further lowering the elevator. They were given 30 sec to make a choice, after which the elevator was raised to block the choice tubes, and the number of flies on each side counted. Typically, all the flies cleared the elevator during the 30 sec duration of each test; where a few remained, they were not included in the calculations. Fig. 2*b* shows the responses of wild-type flies in the temperature choice paradigm. Flies strongly chose the 22°C arm of the chamber.

Effects of mutations on the temperature response. We used the temperature gradient (Fig. 1*a*) to screen 55 extant mutant lines that exhibit different sensory, behavioral, and morphological defects. The majority gave responses that were very similar to wild-type controls (data not shown), suggesting that thermosensation is an essentially independent mechanism. However, two mutants proved particularly interesting.

The first mutant, *bizarre* (*biz*), was also coincidentally the first one isolated in the original countercurrent phototaxis screen (35). No obvious differences in external morphology were observed between *biz* and wild-type flies. When temperature preference was tested in *biz* flies, they exhibited no preference, distributing randomly across the gradient (Fig. 3*b Left*). We next asked whether *biz* flies are completely insensitive to temperature. To address this question, flies were tested on a higher level temperature gradient, over the range 23–36.5°C, as opposed to the standard 18–31.5°C gradient used for screening. On such a gradient, wild-type flies aggregated toward the 23°C end (Fig. 3*a Right*). However, *biz* flies distributed randomly across this gradient as well (Fig. 3*b Right*), indicating that they are completely thermoblind. We have tested this mutant on gradients going up to 45°C and

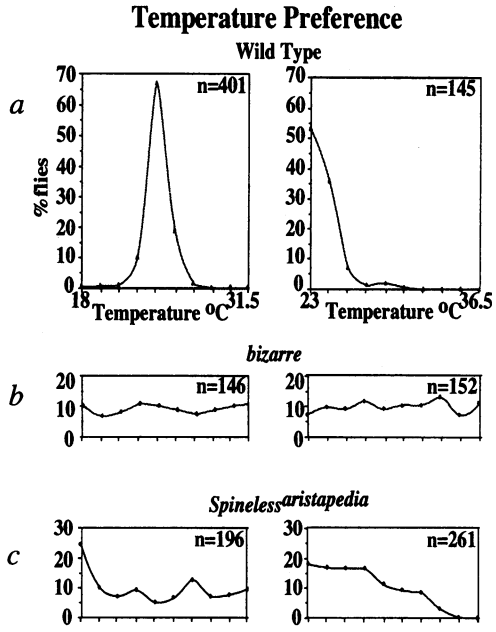

FIG. 3. Temperature gradient responses of wild-type and the mutants *bizarre* and *spineless*^{aristapedia}. (*a*) On the standard 18–31.5°C gradient (*Left*), wild-type flies exhibited a strong preference, peaking at 24°C; on the 23–36.5°C high temperature gradient (*Right*), they aggregated toward the 23°C end, with a marked drop-off at higher temperatures. (*b*) *bizarre* distributed randomly irrespective of the temperature range. (*c*) *spineless*^{aristapedia} distributed broadly on the standard gradient; on the high temperature gradient, the distribution fell off at 33°C, with no flies crossing 35°C. *n* = total number of flies tested for each condition.

observed the same bizarre behavior. Some flies would actually die instead of moving away from the high temperature (data not shown).

A second mutant that showed abnormal behavior on the temperature gradient was the third-chromosomal homeotic mutant *spineless*^{aristapedia} (*ss*^{a}). In this mutant, the aristae and the distal regions of the 3rd antennal segments are transformed into leg-like structures (36). When *ss*^{a} were tested on the standard 18–31.5°C temperature gradient, they exhibited no preference, distributing almost randomly (Fig. 3*c Left*). To determine whether *ss*^{a} flies are completely insensitive to temperature, they were tested on the 23–36.5°C high temperature gradient. In marked contrast to *biz*, the distribution of *ss*^{a}, although broad, dropped off at 33°C, with no flies going beyond 35°C (Fig. 3 *b Right* and *c Right*), demonstrating that *ss*^{a} mutants are only partially thermoinsensitive. Therefore, *ss*^{a} and *biz* have different degrees of defects in thermosensation; whereas *ss*^{a} is only partially insensitive to temperature, in *biz* sensitivity seems to be eliminated altogether.

In Fig. 4 we show the responses of wild-type and *biz* flies in the temperature choice paradigm (Fig. 2*a*). Consistent with their behavior on the thermal gradient, wild-type flies strongly

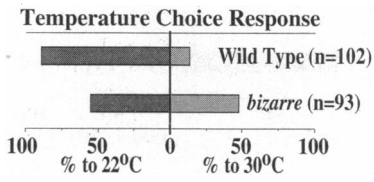

FIG. 4. Temperature choice responses of wild-type and *bizarre* flies. Wild-type flies strongly chose the 22°C arm of the chamber. *bizarre* flies, on the other hand, distributed randomly between 22°C and 30°C. *n* = total number of flies of each genotype.

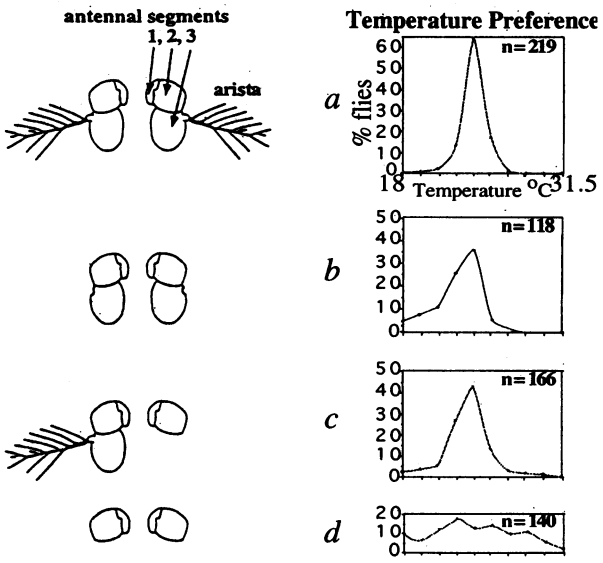

FIG. 5. Effects of ablations on the temperature gradient responses of wild-type *Drosophila*. (*a*) Intact controls exhibited a preference peak at 24°C. (*b*) Removal of both aristae had little effect. (*c*) Flies with a third antennal segment and arista removed from one side also behaved normally. (*d*) Bilateral removal essentially eliminated the response. *n* = total number of flies tested for each condition.

chose the 22°C arm of the chamber, whereas *biz* mutants distributed randomly between the two temperatures.

Effects of ablations on the temperature response. Ablation experiments were used to obtain evidence on the anatomical location of thermoreceptors. When wild-type flies with bilateral ablation of the aristae were tested on the thermal gradient, they exhibited a temperature preference similar to that of intact controls (Fig. 5 *a* and *b*). The same was observed after unilateral removal of a third antennal segment and arista (Fig. 5*c*). However, flies with bilateral removal of the third antennal segments and aristae exhibited no temperature preference, distributing broadly across the gradient (Fig. 5*d*). These experiments indicate that thermoreceptors are located in the third antennal segments, but not in the aristae.

Responses to Humidity. *Humidity choice paradigm.* To investigate hygrosensation, flies were given a choice between moist and dry air in a choice-chamber apparatus adapted for

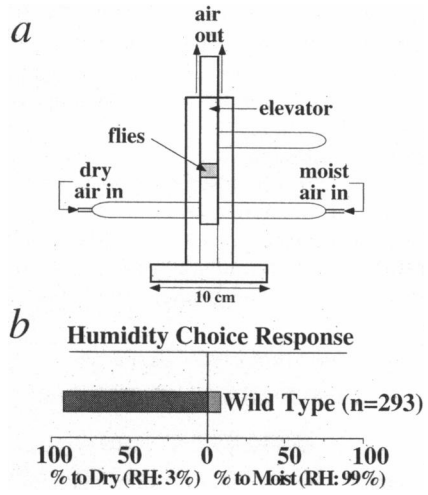

FIG. 6. Humidity choice paradigm. (*a*) Binary choice-chamber apparatus for testing hygrosensation. (*b*) Humidity choice responses of wild-type *Drosophila*. When given a choice between 99% and 3% relative humidity, flies consistently chose the dry arm of the chamber. Data pooled from 293 flies, about 10 flies per test. RH, relative humidity.

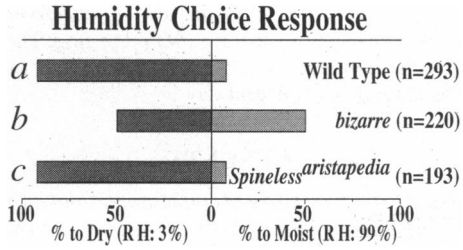

FIG. 7. Humidity choice responses of wild type and the mutants *bizarre* and *spineless*^{aristapedia}. (*a*) Wild-type flies consistently chose the dry arm of the chamber. (*b*) *bizarre* distributed randomly between dry and moist. (*c*) *spineless*^{aristapedia} behaved identically to wild type. *n* = total number of flies of each genotype. RH, relative humidity.

air flow (Fig. 6*a*). In these tests, both streams of air were at room temperature (22°C). Each test involved a group of 10 flies that were tested as described above for the temperature choice paradigm. The relative humidities used were 99% versus 3%. Under these conditions, wild-type flies strongly chose the dry side of the testing chamber (Fig. 6*b*). Perttunen and Salmi (20) found that, when given a choice between two relative humidities, one greater than 87% and the other a lower value, *Drosophila* tended toward the drier alternative. On the other hand, relative humidities less than 77% were preferred over dryer values. Therefore, it appears that a very highly moist atmosphere is aversive to *Drosophila*. Our paradigm, designed for the screening of mutants, provides a sharp distinction between dry and moist extremes, eliciting a clear-cut response.

Effects of mutations on the humidity response. The majority of extant mutants tested for humidity choice behaved just like wild-type controls (data not shown). Here, we present the humidity responses of two mutants that behaved abnormally on the temperature gradient. *biz* flies exhibited no humidity preference, distributing randomly between dry and moist (Fig. 7*b*). Hence, *biz* seem to be blind to humidity as well as temperature. *ss*^{a}, on the other hand, consistently chose the dry environment (Fig. 7*c*). It is manifest, therefore, that the *ss*^{a} and *biz* mutations differentially affect thermosensation and hygrosensation; while both affect thermosensation, though to different extents, only *biz* affects hygrosensation.

Effects of ablations on the humidity response. Selective ablations were used to investigate the location of hygroreceptors. Flies with unilateral ablation of an arista showed no change in behavior relative to intact controls, choosing the dry side of the testing chamber (Fig. 8 *a* and *b*). However, flies with bilateral ablation of the aristae, but with third antennal segments intact, distributed randomly between dry and moist air (Fig. 8*c*). These results indicate that the *Drosophila* arista functions as a hygrometer. Thermoreceptors and hygroreceptors, therefore,

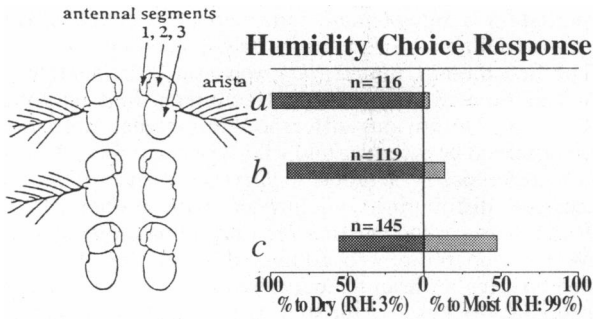

FIG. 8. Effects of ablation on the humidity choice response of wild-type *Drosophila*. (*a*) Intact controls consistently chose the dry side of the chamber. (*b*) Removal of one arista had little effect. (*c*) Bilateral removal of the aristae resulted in a random response. *n* = total number of flies used for each condition.

appear to be in different locations; thermoreceptors in the third antennal segment and hygroreceptors in the arista.

DISCUSSION

The two paradigms described for responses to temperature offer complementary features. The thermal gradient proves to be quite discriminating between wild-type and mutant phenotypes, providing a view of an entire population in regard to the degree of defect in the temperature response. Therefore, we were able to identify *biz* and ss^a as temperature preference mutants, and could distinguish the extents of their defects to show that, whereas ss^a is partially insensitive to temperature, *biz* seems totally temperature-blind (Fig. 3). On the other hand, the temperature choice-chamber provides a rapid, binary test that is well-suited to screening for mutants. Lines established from candidate mutants can then be tested on the temperature gradient for fine grained analysis of their abnormalities. Furthermore, the choice-chamber can be used to test single flies, making it possible to carry out genetic mosaic analysis to identify the foci of defects defined by mutation (40, 41). With its analogous design, the humidity choice paradigm offers the same advantages.

Our studies of the *biz* mutant demonstrate that it is indeed worthy of its name. We have shown here that, whereas the ss^a mutation affects only the temperature response, *biz* abolishes responses to temperature as well as humidity (Figs. 3 and 7). As mentioned earlier, *biz* was originally isolated in a countercurrent screen for non-phototactic mutants (35). Nevertheless, our measurements show that the mutant has a normal electroretinogram. This indicates that the nonphototactic behavior of *biz* is not based on a defect in the first order photoreceptor cells, but rather in higher order information processing by the nervous system. The same may be true for its thermosensory and hygrosensory defects. In any case, this mutant has been useful in demonstrating that the paradigms for screening for mutants can lead to a genetic dissection of the thermosensory and hygrosensory receptors and pathways.

Surgical manipulation enabled us to exclude a role of the arista in the temperature preference response; however, extirpation of the third antennal segments abolished this response (Fig. 5). Thus, in addition to the previously well-known function of the third antennal segment in olfaction (39, 42), our results suggest that it also mediates thermosensation. However, it should be noted that though flies with bilateral ablation of the third antennal segments distributed broadly across the temperature gradient, the distribution did fall off at higher temperatures, approaching zero at 31.5°C (Fig. 5*d*). Therefore, we cannot exclude the presence of high-temperature receptors in other regions of the body. In humans, for example, high temperatures are detected by two separate classes of receptors: (*i*) warm thermoreceptors are activated by temperatures between 32°C and 45°C and (*ii*) thermal pain receptors respond only to temperatures greater than 45°C (21, 22). It is conceivable that flies, too, may possess different types of high temperature receptors. If so, these might be identified by different gene mutations.

The humidity choice tests on surgically manipulated flies indicated that hygroreceptors are housed in the arista (Fig. 8). This constitutes the first attribution of a primary sensory function to this curious organ. An indirect auditory function has been known for a long time; the sail-like movement of the arista in response to sound twists the third antennal segment, thus stimulating Johnston's organ at its base (43). In a detailed anatomical study of the *Drosophila* arista, Foelix *et al.* (44) ruled out a possible chemoreceptive function owing to the absence of pores in the aristal cuticle. In addition, they argued against a role in mechanoreception on the basis of both ultrastructure and projection pathway. The authors speculated that the arista might be involved in thermo- as well as hygrosensation. Our results indicate a role only in hygrosensation. This raises the question about how humidity might affect the aristal neurons. A plausible possibility is that changes in relative humidity alter the conformation of the aristal cuticle, analogous to a hair hygrometer, and these deformations stimulate the underlying sensilla.

Taken together, the ablation experiments indicate that, in *Drosophila*, thermoreceptors and hygroreceptors are in different locations: the third antennal segment housing thermoreceptors and the arista housing hygroreceptors (Figs. 5 and 8). Based on electrophysiological and morphological data, a number of studies have claimed that a general feature of insect thermo- and hygrosensors is that they are housed within the same antennal sensilla (for reviews, see refs. 27–30). Our results are inconsistent with this generalization.

The localization of hygroreception to the arista raises an intriguing question about the normal hygroresponse of the ss^a mutant. In ss^a, the cuticles of the arista and the distal portion of the third antennal segment are transformed into leg cuticle. Given the transformation of the arista, one might have expected this mutant to be defective in hygrosensation, but that is not the case. A possible explanation is that, despite the transformation of the aristal cuticle, the homeotically transformed structure might still possess certain neurons of aristal identity. In a comparative study of the sensory projections from wild-type versus ss^a antennae, Stocker and Lawrence (45) reported that the majority of ectopic fibers in ss^a behave precisely like antennal and aristal axons in the brain. Two exceptions to this rule were noted. In the antennal glomeruli, the major site of projections from the third antennal segments, terminals were found to be randomly distributed. In addition, there was an ectopic tract of fibers extending into the anterior subesophageal ganglion that is not found in wild-type antennal projections. These similarities and differences between projection patterns, taken in the context of the results we obtained, might provide clues concerning the brain centers involved in thermo- and hygrosensation. For example, the abnormal projection pattern in the antennal glomeruli of ss^a and the abnormal temperature response of this mutant suggest the involvement of this center in thermosensation.

We have demonstrated that *Drosophila* provides a model system for neurogenetic analysis of thermosensation and hygrosensation. This approach, coupled with mosaic and electrophysiological analyses, opens up the avenue to address the following questions: What are the neuronal identities of the thermosensors and hygrosensors? How do heat and humidity act on these neurons to alter membrane polarization? How are quantitative aspects of the stimuli encoded electrically? And what neural networks are involved in information processing and response?

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