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Olfactory Discrimination of Species, Sex, and Sexual Maturity by the Hermann's Tortoise *Testudo hermanni*

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Many animals obtain reliable information about potential mates, including whether they are conspecific, sexually mature, and healthy or not, mostly from olfactory cues. Previous experiments with snakes and lizards have shown that individuals can recognize conspecifics, sex, mating status, and health condition of potential partners by chemical cues. Using choice experiments, we examined whether both male and female Hermann's Tortoises, *Testudo hermanni*, can detect and distinguish the odor of conspecifics from that of another species and an odorless control, and are able to discriminate sex and sexual maturity of individuals by chemical cues. We found that both sexes correctly discriminated between their own species and another species' odors, but only males can distinguish sex and sexual maturity of potential mates by olfactory cues. These results indicate a sexual dimorphism in olfactory sensitivity in this species that might be derived from sexual selection and suggest that males and females should rely on different communication channels during social interactions.

CHEMICAL communication is used by vertebrates in many contexts and may be especially important when visual and auditory communication is restricted or impossible (Stoddard, 1980; Wyatt, 2003). Pheromones play an important role in the intraspecific communication of many reptiles, mainly lizards and snakes (Chiszar et al., 1990; Alberts and Werner, 1993; LeMaster et al., 2001; López and Martín, 2002; López et al., 2002), with a variety of functions (Halpern, 1992; Mason, 1992; Cooper, 1994). For example, chemical signals may permit discrimination between males and females, or between neighbor and non-neighbor males, which could help to stabilize social systems (Bull et al., 2000; Aragòn et al., 2001). Some reptiles may detect and follow scents passively laid on the substrate by conspecifics to locate mating partners (Ford, 1986; Bonnet et al., 1999). Pheromones have been found to not only communicate the sex, but also to contain information concerning the species identification, body size, relatedness, or sexual attractiveness of the scent producer (Cooper and Pèrez-Mellado, 2002; Gonzalo et al., 2004).

Although communication by chemical cues has been documented in chelonian species as well (Neill, 1948; Legler, 1960; Rose, 1970), little attention has been devoted to the role that odor and olfactory stimuli play in tortoise behavior. In fact, olfactory communication occurs in diverse social contexts, including aggregations, aggression, combat, and sexual behavior (Manton, 1979; Mason, 1992). Chelonians have well-developed nasal olfactory and vomeronasal systems (Halpern, 1992; Hatanaka and Matsuzaky, 1993) that enable them to perceive a vast array of odors (e.g., many tortoises are attracted by flavorful fruits and vegetables, Frye, 1995) and to modulate their social behavior (Mason, 1992; Graham et al., 1996; Quinn and Graves, 1998; Muñoz, 2004). Moreover, all chelonian families, with the exception of Testudinidae, have two pairs of glands located between the anterior and the posterior edges of the plastral bridges that, in some species, exude a liquid with a strong musky odor when animals are distressed (Kuchling, 1999). Odor other than that of the glands near the bridges may be important during social encounters, since sniffing is also often directed to the tail or the cloaca. Secretions of glands near the bridge may play a role in aggressive encounters between male tortoises and may be important in mediating courtship behavior (Weaver, 1970; Coombs, 1977; Luckenbach, 1982), but their precise function is still poorly known, and the substances are not well characterized.

The Hermann's Tortoise, one of the three testudinid species endemic to Europe, maintains nonexclusive home ranges and exhibits a promiscuous mating system with multiple copulations. The breeding season has two activity peaks in April–May and in September–October (Willemsen and Hailey, 2003). Sexual dimorphism is noticeable: males are smaller than females and their activity and mobility is higher than that of females, which suggests that males allot more energy to movement than to growth (Berry and Shine, 1980). Both sexes have a strong site tenacity (Chelazzi and Francisci, 1979) and show a prompt homing behavior based on olfaction, although females appear to have much lower homing ability than males (Chelazzi and Delfino, 1986). Courtship behavior in Hermann's Tortoise is based on a multiple signaling system that includes olfactory, visual, tactile, and acoustic signals as in other testudinids (Galeotti et al., 2005a). Although sniffing occurs in many encounters, it is particularly important during courtship phases in spring, but the function of chemical cues remains unknown, as do the nature of substances secreted and the organs that produce them. Undoubtedly, a strong musky odor emanates from femoral and tail regions, which are the body parts usually sniffed during social interactions.

In this study, we experimentally tested the hypothesis that Hermann's Tortoises are able to recognize chemical cues from conspecifics and to modify their behavior in response to such cues. Specifically, by means of two-choice experiments, we investigated whether males and females of this species are able to discriminate species, sex, and sexual maturity of potential partners.

Materials and Methods

We conducted this study during spring and summer 2002–2003, at the "CARAPAX" European Centre for Tortoise Conservation, located at Massa Marittima (Tuscany, Central Italy), where 8,000 individuals of several tortoise species reproduce in enclosures, in semi-natural conditions and at high density. Tortoises are accustomed to humans and most individuals do not modify their behavior when approached.

General design of experiments.—Two-choice experiments were conducted from 10 May to 25 June 2002 and from 3 May to 30 May 2003, between 0800 and 1900 hrs in a large enclosure (650 m^2) where Hermann's Tortoises reproduced freely. For each experiment (see below), we used 20 different males and 20 different females, for a total of 200 individuals, all coming from a captive Tuscan population. Tested animals were all sexually mature (as determined by carapace length, males >12 cm, females >14 cm, Willemsen and Hailey, 1999), and experiments were conducted only while tortoises were courting in the enclosure, during calm dry days, and at ambient temperatures ranging from 24 to 30 C.

In all experiments, the individual being tested, either male or female, was cautiously approached by the same observer (R. S., who fed the tortoises during the dates of the experiment) and presented simultaneously, ca. 2 cm anterior to its nose, a pair of plastic opaque syringes (Fig. 1) that contained different olfactory stimuli depending on the experiment type (see below). This method is derived from the well-known technique of cotton-tipped swabs used for assessing responses by squamate reptiles to chemical stimuli (see Cooper, 1998 for a thorough review of this method). Apart from the second control experiment (see below), all olfactory stimuli came from a sample of tortoises (120 males and 120 females) not used in the experiments and maintained in a different enclosure. Olfactory stimuli were obtained by rubbing a cotton swab against the body parts normally sniffed by tortoises during social encounters, i.e., the femoral and cloacal regions. Swabs were then inserted into the syringes, so that chemical stimuli were invisible or visually identical to the animals being tested; syringes were changed in each trial. The observer was blind to treatments, and we recorded the number of sniffs directed toward a certain syringe within 2 min from stimuli presentation in order to determine potential discrimination between stimuli. "Sniffing" behavior is widespread in chelonians and typically consists of extending the neck and lowering the head while approaching nostrils to the object of interest (Carpenter and Ferguson, 1977). If the tested tortoise did not respond at all during the control period, the test was repeated the next day.

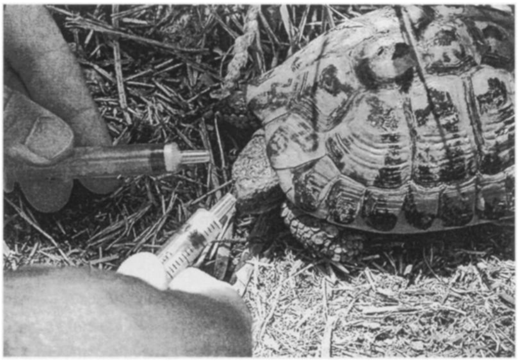

Fig. 1. Method of presenting olfactory stimuli to focal tortoises. Swabs containing chemical stimuli are inside the syringes.

Control experiments.—In the first experiment we checked the effectiveness of syringes in diffusing odors by using a cotton swab dipped in distilled water, which served as an odorless control for responses to the experimental setting, and another cotton swab dipped in peach juice. Forty individuals were used (20 males and 20 females).

In the second experiment, we tested the effectiveness of cotton swabs in collecting odor from tortoises by comparing responses of 40 individual (20 males and 20 females) to a pair of syringes containing a cotton swab with tortoise odor and a cotton swab dipped in distilled water as an odorless control, respectively. We collected olfactory stimuli from the individual used in this experiment and, to avoid pseudo-replication, we used a cross-designed stimulation, the first male in the sequence being tested with the odor of the first female and vice versa, so that in each of the 40 trials we used a different stimulus.

Discrimination experiments.—We first analyzed whether odors collected from femoral and cloacal regions of tortoises transmitted information useful for species recognition by testing 20 males and 20 females with two olfactory stimuli: the odor of their own species (*T. hermanni*) and the odor collected from femoral and cloacal regions of another sympatric species, *Testudo graeca*, which shares the same habitat types and exhibits the same sniffing behavior during social interactions. Males were presented a pair of female stimuli, and females a pair of male stimuli. To avoid pseudo-replication we used the odors of 80 different individuals (40 males and 40 females of the two species).

In the second experiment, we checked whether male and female tortoises were able to discriminate between sexes by presenting focal individuals with the paired odors of a mature male and a mature female. To avoid pseudo-replication we used the odors of 80 different individuals (40 males and 40 females).

The third experiment, examining the ability of both sexes to discriminate between reproductive conditions of potential partners, was carried out by simultaneously presenting focal individuals with the odors of a mature and an immature individual of the opposite sex. To avoid pseudo-replication we used the odors of 80 different individuals whose maturity was determined by carapace length (mature males: 145 ± SE 3.8 mm, range: 120–179 mm, $n = 20$; immature males: 108.5 ± SE 1.3 mm, range: 94–118 mm, $n = 20$; mature females: 165.9 ± SE 2.9 mm, range 145–194 mm, $n = 20$; immature females: 118.1 ± SE 3.8 mm, range: 94–136 mm, $n = 20$). To determine whether individuals discriminated between different stimuli, we used the Wilcoxon test for paired samples in order to compare the number of sniffs directed by males and females toward each stimulus in each experiment.

Results

Control experiments.—All 40 tortoises but one sniffed exclusively at the syringe containing the peach juice odor while ignoring the syringe containing the odorless stimulus ($Z = -5.53$, $P < 0.001$, $n = 40$). In the second control experiment, 29 individuals (14 males and 15 females) sniffed only the syringe diffusing tortoise odor compared to 11 individuals that sniffed only the odorless syringe ($Z = -4.67$, $P < 0.001$, $n = 40$).

Discrimination experiments.—Both male and female Hermann's Tortoises discriminated between conspecific and heterospecific olfactory stimuli, smelling the syringe diffusing the odor of their own species more times ($Z = -3.6$, $P < 0.001$ for males, and $Z = -3.4$, $P < 0.001$ for females, $n = 20$ for both sexes, Fig. 2A). Males were also able to discriminate sex by odor, sniffing the syringe diffusing female scent more frequently than the one diffusing male scent ($Z = -3.58$, $P < 0.001$, $n = 20$, Fig. 2B), while sniff rates of females did not differ between male and female scents ($Z = -0.80$, $P = 0.42$, $n = 20$, Fig. 2B). Similarly, males discriminated between mature and immature females by sniffing ($Z = -3.30$, $P < 0.001$, $n = 20$, Fig. 2C), while females showed only a non-significant tendency to sniff odors of mature males more than those of immature males ($Z = -1.71$, $P = 0.09$, $n = 20$, Fig. 2C).

Discussion

This study showed that surface chemicals from femoral and tail regions of Hermann's Tortoises represent olfactory stimuli that both sexes detect and discriminate from an odorless control. Moreover, these chemicals may convey information on species, sex, and sexual maturity of individuals, as for instance occurs in the Desert Tortoise (*Gopherus agassizii*), whose chin gland secretion seems to play a role in recognition of partners or competitors during the mating season (Alberts et al., 1994; Bulova, 1997). Our experiments indicate that olfactory communication is potentially important in tortoise sexual behavior, as occurs in other turtles (Grassman, 1993; Graham et al., 1996; Quinn and Graves, 1998; Muñoz, 2004). For example, experimental elimination of olfactory function resulted in a 60–70% reduction in reproductive behavior in European Pond Turtles, *Emys orbicularis* (Boiko, 1984).

During the breeding season, both sexes are able to discriminate conspecifics by sniffing; discrimination between pheromones of conspecific and heterospecific individuals is clearly beneficial for both sexes, since *T. hermanni* is sympatric with *T. graeca* and *T. marginata* in the eastern parts of its range (the Balkan peninsula), where they share the same habitat types. Thus,

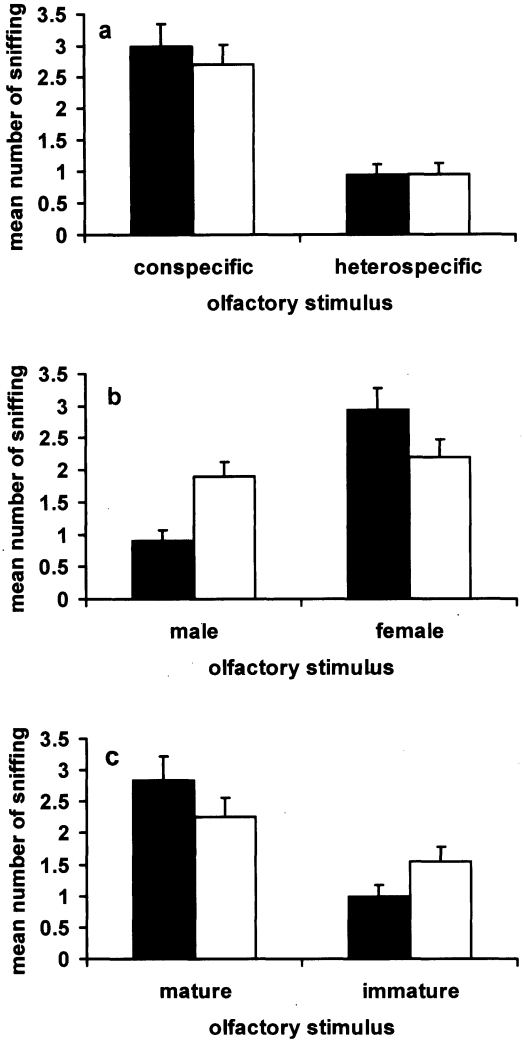

Fig. 2. Preferences exhibited by male (black bars) and female (blank bars) Hermann's Tortoises toward different olfactory stimuli: (A) conspecific vs. heterospecific odor; (B) odor of same vs. opposite sex; and (C) mature vs. immature individual odor. Error bars represent 1 SE.

males might use olfactory cues to avoid time-consuming searches for inappropriate potential mates, and females might refuse interspecific courtship based on odor. However, only males appear to discriminate sex and sexual maturity by scent. Thus, interestingly enough, males are more responsive to olfactory stimuli than females, which confirm previous findings of reduced olfactory responses in female Hermann's Tortoises (Chelazzi and Delfino, 1986). This sexual difference in olfactory discrimination may derive from mating strategies and life history of the species under study. Indeed, the absence of sex discrimination in females might simply reflect the lack of a need to locate mates by scent-trailing (Cooper and Vitt, 1986) rather than a true lack of ability to discriminate. Females may rely more on other communication channels based on visual, tactile, and acoustic signals (Sacchi et al., 2004; Galeotti et al., 2005b). In fact, although we have neither direct behavioral evidence nor literature data for trailing of females by males in tortoises, in many chelonian species, and particularly in testudinids, males are the ones that search for dispersed females at the time of breeding, whereas females have a passive role in mate choice (Berry and Shine, 1980; Kuchling, 1999; Muñoz, 2004; P. Galeotti and R. Sacchi, pers. obs.). Therefore, the sexual dimorphism in olfactory performance might be sexually selected if males actually possess chemo-orientation ability to locate dispersed females during their period of sexual receptivity, and this ability gives them a significant fitness advantage over other males, minimizing wasted time and energy in pursuing inappropriate or unavailable mates. Moreover, the ability to discriminate and avoid chemical stimuli from other males could potentially reduce costly agonistic encounters during the breeding season. Further study is needed to assess scent-trailing ability and ability to detect conspecific odors from a distance.

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Literature Cited

Alberts, A. C., D. C. Rostal, and V. A. Lance. 1994. Studies on the chemistry and social significance of chin gland secretions in the Desert Tortoise, *Gopherus agassizii*. Herpetological Monographs 8:116–124.

Alberts, A. C., and D. I. Werner. 1993. Chemical recognition of unfamiliar conspecifics by Green Iguanas: functional significance of different signal components. Animal Behaviour 46:197–199.

Aragón, P., P. López, and J. Martín. 2001. Chemosensory discrimination of familiar and unfamiliar conspecifics by lizards: implication of field spatial relationships between males. Behavioral Ecology and Sociobiology 50:128–133.

Berry, J. F., and R. Shine. 1980. Sexual size dimorphism and sexual selection in turtles (order Testudines). Oecologia 44:185–191.

Boiko, V. P. 1984. The participation of chemoreception in the organization of reproductive behavior in *Emys orbicularis* (Testudines, Emydidae). Zoologichesky Zhurnal 63:584–589.

BONNET, X., D. BRADSHAW, R. SHINE, AND D. PEARSON. 1999. Why do snakes have eyes? The (non-) effect of blindness on Island Tiger Snakes (*Notechis scutatus*). Behavioral Ecology and Sociobiology 46:267–272.

BULL, C. M., C. L. GRIFFIN, E. J. LANHAM, AND G. R. JOHNSTON. 2000. Recognition of pheromones from group members in a gregarious lizard, *Egernia stokesii*. Journal of Herpetology 34:92–99.

BULOVA, S. J. 1997. Conspecific chemical cues influence burrow choice by Desert Tortoises (*Gopherus agassizii*). Copeia 1997:802–810.

CARPENTER, C., AND G. W. FERGUSON. 1977. Variation and evolution of stereotyped behavior in reptiles, p. 335–354. *In*: Biology of the Reptilia, Vol. 7. C. Gans and D. W. Tinkle (eds.). Academic Press, New York.

CHELAZZI, G., AND G. DELFINO. 1986. A field test of the use of olfaction in homing by *Testudo hermanni* (Reptilia: Testudinidae). Journal of Herpetology 20:451–453.

CHELAZZI, G., AND F. FRANCISCI. 1979. Movement patterns and homing behaviour of *Testudo hermanni* Gmelin (Reptilia: Testudinidae). Monitore Zoologico Italiano (N.S.) 13:105–127.

CHISZAR, D., T. MELCER, T. LEE, C. W. RADCLIFFE, AND D. DUALL. 1990. Chemical cues used by prairie rattlesnakes (*Crotalus viridis*) to follow trails of rodent prey. Journal of Chemical Ecology 16:79–86.

COOMBS, E. M. 1977. Population Status, Behavior, and Physiology of the Desert Tortoise, *Gopherus agassizii*, in Southwestern Utah, with Inferences Drawn from Related Desert Ectotherms. Bureau of Land Management, Cedar City, Utah.

COOPER, W. E., JR. 1994. Chemical discrimination by tongue-flicking in lizards: a review with hypotheses on its origin and its ecological and phylogenetic relationships. Journal of Chemical Ecology 20:439–487.

COOPER, W. E., JR. 1998. Evaluation of swab and related tests as a bioassay for assessing responses by squamates reptiles to chemical stimuli. Journal of Chemical Ecology 24:841–866.

COOPER, W. E., JR., AND V. PÈREZ-MELLADO. 2002. Pheromonal discrimination of sex, reproductive condition, and species by the Lacertid Lizard *Podarcis hispanica*. Journal of Experimental Zoology 292:523–527.

COOPER, W. E., JR., AND L. J. VITT. 1986. Tracking of female conspecific odor trails by male Broad-headed Skinks (*Eumeces laticeps*). Ethology 71:242–248.

FORD, N. B. 1986. The role of pheromone trails in sociobiology of snakes, p. 261–278. *In*: Chemical Signals in Vertebrates, Vol. 4. D. Duvall, D. Müller-Schwarze, and R. M. Silverstein (eds.). Plenum Publishing, New York.

FRYE, F. L. 1995. Nutritional consideration, p. 82–97. *In*: Health and Welfare of Captive Reptiles. C. Warwick, F. L. Frye, and J. B. Murphy (eds.). Chapman & Hall, London.

GALEOTTI, P., R. SACCHI, M. FASOLA, D. PELLITTERI ROSA, M. MARCHESI, AND D. BALLASINA. 2005a. Courtship displays and mounting calls are honest, condition-dependent signals that influence mounting success in Hermann's Tortoises. Canadian Journal of Zoology 83:1306–1313.

GALEOTTI, P., R. SACCHI, D. PELLITTERI ROSA, AND M. FASOLA. 2005b. Female preference for fast-rate, high-pitched calls in Hermann's Tortoises *Testudo hermanni*. Behavioral Ecology 16:301–308.

GONZALO, A., C. CABIDO, J. MARTÍN, AND P. LÓPEZ. 2004. Detection and discrimination of conspecific scents by the anguid slow-worm *Anguis fragilis*. Journal of Chemical Ecology 30:1565–1572.

GRAHAM, T., A. GEORGES, AND N. MCELHINNEY. 1996. Terrestrial orientation by the Eastern Long-Necked Turtle, *Chelodina longicollis*, from Australia. Journal of Herpetology 30:467–477.

GRASSMAN, M. 1993. Chemosensory orientation behavior in juvenile sea turtles. Brain Behavior and Evolution 41:224–228.

HALPERN, M. 1992. Nasal chemical senses in reptiles: structure and functions, p. 423–522. *In*: Biology of the Reptilia. Brain, Hormones, and Behavior, Vol. 18. C. Gans and D. Crews (eds.). University of Chicago Press, Chicago.

HATANAKA, T., AND O. MATSUZAKY. 1993. Odor responses of the vomeronasal system in Reeve's Turtle *Geoclemys reevesii*. Brain Behavior and Evolution 23:183–186.

KUCHLING, G. 1999. The Reproductive Biology of the Chelonia. Springer-Verlag, Berlin Heidelberg.

LEGLER, J. M. 1960. Natural history of the Ornate Box Turtle, *Terrapene ornata ornata* Agassiz. Publication of the Museum of Natural History, University of Kansas 11:527–669.

LEMASTER, M. P., T. MOORE, AND R. T. MASON. 2001. Conspecific trailing behaviour of Red-Sided Garter Snakes, *Thamnophis sirtalis parietalis*, in natural environment. Animal Behaviour 61:827–833.

LÓPEZ, P., AND J. MARTÍN. 2002. Chemical rival recognition decreases aggression levels in male Iberian Wall Lizards, *Podarcis hispanica*. Behavioral Ecology and Sociobiology 51:461–465.

LÓPEZ, P., A. MUÑOZ, AND J. MARTÍN. 2002. Symmetry male dominance and female mate preferences in the Iberian Rock Lizard, *Lacerta monticola*. Behavioral Ecology and Sociobiology 52:342–347.

LUCKENBACH, R. A. 1982. Ecology and management of the Desert Tortoise (*Gopherus agassizii*) in California, p. 1–37. *In*: North American Tortoises: Conservation and Ecology. R. B. Bury (ed.). U.S. Fish and Wildlife Service, Research Report 12, Washington, D.C.

MANTON, M. L. 1979. Olfaction and behavior, p. 289–301. *In*: Turtles: Perspectives and Research. M. Harless and H. Morlock (eds.). Wiley and Sons, New York.

MASON, R. T. 1992. Reptilian pheromones, p. 114–228. *In*: Biology of the Reptilia. Brain, Hormones, and Behavior, Vol. 18. C. Gans and D. Crews (eds.). University of Chicago Press, Chicago.

MUÑOZ, A. 2004. Chemo-orientation using conspecific chemical cues in the Stripe-Necked Terrapin (*Mauremys leprosa*). Journal of Chemical Ecology 30:519–530.

NEILL, W. T. 1948. Odor of young box turtles. Copeia 1948:130.

QUINN, V. S., AND B. M. GRAVES. 1998. Home pond discrimination using chemical cues in *Chrysemys picta*. Journal of Herpetology 32:457–461.

ROSE, F. L. 1970. Tortoise chin gland fatty acids composition: behavioral significance. Comparative Biochemistry and Physiology 32:577–580.

SACCHI, R., P. GALEOTTI, M. FASOLA, AND D. BALLASINA. 2004. Vocalizations and courtship intensity correlate with mounting success in Marginated Tortoises *Testudo marginata*. Behavioral Ecology and Sociobiology 55:95–102.

STODDARD, M. D. 1980. The Ecology of Vertebrate Olfaction. Chapman and Hall, London.

WEAVER, W. G., JR. 1970. Courtship and combat behavior in *Gopherus berlandieri*. Bulletin of the Florida State Museum 15:1–43.

WILLEMSEN, R. E., AND A. HAILEY. 1999. Variation of body size of the tortoise *Testudo hermanni* in Greece: proximate and ultimate causes. Journal of Zoology (London) 248:379–396.

WILLEMSEN, R. E., AND A. HAILEY. 2003. Sexual dimorphism of body size and shell shape in European tortoises. Journal of Zoology (London) 260:353–365.

WYATT, T. D. 2003. Pheromones and Animal Behaviour. Cambridge University Press, Cambridge.

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