DO MOUNTING VOCALISATIONS IN TORTOISES HAVE A COMMUNICATION FUNCTION? A COMPARATIVE ANALYSIS

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We review the occurrence of vocalisations in tortoise courtship in order to investigate their functions, if any, taking into account evolutionary history, habitat and body size. Courtship behaviour has been described for 101 (41%) of all chelonian species. Among them, mount vocalisations occur in 35 species belonging to families Testudinidae Batsch, 1788 (*n*=29), Trionychidae Fitzinger, 1826 (*n*=3), Emydidae Rafinesque, 1815 (*n*=2), and Bataguridae = Geoemydidae Theobald, 1868 (including Batagurinae Gray, 1869) (*n*=1). The mapping of vocalisation evolution along the phylogenetic tree revealed that mount vocalisations are an ancestral trait, being present from the origin of Cryptodira, and calls mainly occur in terrestrial or semi-terrestrial species. In the species and subspecies of Testudinidae we considered, mounting-calls show an harmonic structure with frequency and amplitude modulation, which are acoustic features not compatible with mechanisms of sound production based simply on air flow through respiratory tracts. Moreover, the call fundamental frequency was negatively related to body size. Since in birds and amphibians such size-frequency relationship is due to a correlation existing between body size and mass of the vibrating device used to produce sounds (a greater device produces longer wavelengths and consequently low frequencies), in tortoises vocalisations might also be produced by vibrating structures not yet described. All these findings strongly suggest that mounting-calls might have the function to convey information on the signaller to conspecifics, possibly influencing the outcome of sexual interactions.

Key words: body size, call frequency, chelonians, mount vocalisations, phylogeny

INTRODUCTION

The courtship and copulatory behaviour of the chelonia is elaborate, and based on a multiple signalling system involving visual, olfactory, and acoustic signals. Particularly interesting are the vocalisations associated with mounting, as this is the predominant – or for some species the only – behaviour during which turtles vocalise (Ernst & Barbour, 1989). Despite this, chelonian vocalisations have been studied only occasionally (Campbell & Evans, 1967, 1972; Mrosovsky, 1972; see also Gans & Maderson, 1973 for a review), and almost nothing is known about their production and function (Berry & Shine, 1980; Swingland & Stubbs, 1985; Olsson & Madsen, 1998). The lack of interest in the acoustic behaviour of chelonians may have resulted from the general belief among herpetologists that turtles and tortoises lack a functional sense of hearing (e.g. Pope, 1955), but at present we know from many studies that a number of species have a considerable auditory sensitivity to sounds below 1000 Hz (Wever & Vernon, 1956*a,b*; Gulick & Zwick, 1966; Patterson, 1966; Campbell & Evans, 1967; Ridgway *et al.* 1969), and this no doubt enables the animal to perceive many acoustic signals both on land and in water. Therefore, the expla-

nation proposed by many authors that tortoise vocalisations are simple "noises" involuntarily produced by copulatory movements (Weaver, 1970; Mrosovsky, 1972) sounds very unlikely. In a recent study on *Testudo marginata* Schoepff, 1793 (Sacchi *et al.* 2003), we showed that two features (call rate and duration) of male calls strongly correlated with male mounting success (number of effected mounts and number of mounted females by a male). Also, in *Testudo hermanni* Gmelin, 1789 we found that different call features (i.e. calling rate, frequency range, call duration) were significantly related to various aspects of male quality (weight and body size, general health condition, and mounting success, P. Galeotti & R. Sacchi unpublished data), suggesting a possible adaptive role of vocalisations in conveying information about male general quality in socio-sexual contexts.

In addition, we found that call fundamental frequency of both species was negatively related to male body size (weight, head and carapace length). This last finding suggests that sound production in tortoises might be due to a specific vibrating structure, since in both leptodactyline frogs and birds, which use vocal cords and the syrinx for generating sounds, the fundamental frequency of vocalisations similarly decreases with increasing body size (Ryan, 1985; Ryan & Brenovitz, 1985). In fact, body size affects sounds by determining the size of vibrating structures producing them, and animals can most effectively produce sounds

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TABLE 1. Number of males and acoustic features of calls (mean ± SE) for the species of Testudinidae recorded.

Species	No. individuals	No. vocalizations	Duration (ms)	Gap between calls (s)	Fundamental frequency (Hz)
Geochelone chilensis = *Chelonoidis chilensis* (Gray, 1870)	1	12	278±23	2.15±0.12	209.2±2.1
Geochelone carbonaria = *Chelonoidis carbonaria* (Spix, 1824)	2	52	84±2	0.29±0.02	109.9±0.4
Geochelone denticulata = *Chelonoidis denticulata* (Linnaeus, 1766)	1	3	907±19	1.77±0.32	230.0±18.6
Geochelone sulcata (Miller, 1779)	2	44	255±9	1.47±0.09	114.0±4.0
Geochelone radiata = *Astrochelys radiata* (Shaw, 1802)	1	53	248±2	1.03±0.02	115.8±0.4
Testudo kleinmanni Lortet, 1883	1	98	464±18	0.48±0.02	516.3±0.4
Testudo h. hermanni Gmelin, 1789	40	2196	144±6	2.4±0.1	602.1±17.1
Testudo h. boettgeri Mojsisovics, 1889	2	48	209±18	2.86±0.12	535.3±79.8
Testudo graeca graeca Linnaeus., 1758	13	789	462±49	2.1±0.2	391.3±20.1
Testudo marginata Schoepff, 1793	19	1479	475±4	1.6±0.1	295.9±1.0
Indotestudo travancorica (Boulenger, 1907)	1	2	483±17	—	400.0±20.0

with wavelengths approximately equal to or smaller than their body size (Bradbury & Vehrencamp, 1998).

Therefore, the aims of the present study are to review the courtship behaviour of chelonians in order (1) to explore the occurrence of mounting-calls and other vocalisations in this taxon by taking into account the evolutionary history and breeding habitat; and (2) to analyse the effects of body size on call frequency in a sample of calling species.

If tortoise calls are produced by a specific vibrating structure and function to convey information about the signaller to conspecifics, we may expect that (1) mounting-calls should mainly occur in species where an acoustic communication system based on air flow is more likely to be developed and may confer some advantages to signallers, i.e. in terrestrial or semi-terrestrial species; aquatic species of chelonians mate underwater (Ernst & Barbour, 1989; Kaufmann, 1992) and respiratory exchanges needed to produce sounds are unlikely; (2) the mounting-call should have a complex structure, showing harmonics and both frequency and amplitude modulation; these acoustic features are unique to vibrating systems, and are not present in sounds produced by simple air flow through larynx (Bradbury & Vehrencamp, 1998). For example, the hiss emitted by rattlesnakes (*Crotalus adamanteus*), which is a consequence of body inflation being produced only during the periods of rapid inhalation, is a simple sound without a distinct amplitude and frequency modulation and harmonics (Kinney *et al.* 1998); (3) given the size-frequency relationship peculiar of sounds produced by vibrating structures (Ryan & Brenovitz, 1985; Ryan, 1985), call fundamental frequency should decrease with increasing body size among tortoises species; (4) call features should be related to some individual qualities increasing the signaller success in socio-sexual contexts. This last prediction was already verified in *Testudo marginata* (Sacchi *et al.* 2003) and *T. hermanni* (P. Galeotti & R. Sacchi, unpublished data), thus we focused on the first three predictions in this study.

METHODS

Information about courtship behaviour of chelonians as a whole was collected from the most comprehensive review of the taxon (Ernst & Barbour, 1989). For each of the 246 recognised species we recorded courtship behaviour, habitat (marine, fresh-water, semi-terrestrial, and terrestrial) and the occurrence of mounting-calls. Since vocalisations are the most striking trait of tortoise's mating, we considered a species as not vocalising if calls were not explicitly reported in the courtship description. We also recorded the occurrence of vocalisation outside sexual contexts.

The acoustic structure of vocalisations was examined by recording 4776 mounting-calls of 83 males belong-

ing to 11 different species and sub-species of Testudinidae (Table 1) during spring-summer 2001-2002, at the European Centre for Tortoise Conservation ("CARAPAX", Massa Marittima, Tuscany, Central Italy), where 8000 individuals of several chelonian species reproduce in enclosures, in semi-natural conditions. We recorded the vocalisations using a Sony TCD-D7 DAT tape recorder connected to a SHURE shotgun microphone with a hypercardioid capsule. For each recorded species (or subspecies) we considered the carapace length as a measure of body size; we took the mean carapace length for species with many recorded individuals. *Testudo hermanni hermanni* Gmelin, 1789 and *T. hermanni boettgeri* Mojsisovics, 1889 were considered separately due to their difference in mean carapax size (14 and 19 cm respectively, Ernst & Barbour, 1989).

Recordings obtained from each male during courtship were analysed by the AVISOFT SAS-LAB pro software (©Specht, 1993). The best resolution was achieved analysing vocalisations in the 0-4 kHz frequency range, with a sampling rate of 8000 sample/s, frequency resolution 15 Hz and time resolution 8 ms. For each call we selected and measured directly on the screen the fundamental frequency in Hz; for species with many recorded individuals, we used the mean fundamental frequency.

PHYLOGENETIC ANALYSES AND STATISTICS

We produced two phylogenetic trees using the TreeEdit program (Rambaut & Charleston, 2001): the first included all chelonian genera encompassing vocal species and was used to trace vocalization appearance in the evolutionary history of chelonians (Fig. 1). This phylogeny was based on the molecular information by Shaffer *et al.* (1997), derived from cythocrome *b* and 12S ribosomal DNA sequences, combined with the phylogenetic trees on Chelidae Gray, 1825 from Seddon *et al.* (1997) and Georges *et al.* (1998, 2002); on Emydidae from Bickham *et al.* (1996), Lamb & Osentovsky (1997), Lindeman (2000) and Feldman & Parham (2002); and on Bataguridae from Parham *et al.* (2001). In the few cases where there was no completely bifurcating phylogeny available, we formed polytomies (nodes with more than two descendant taxa).

The second tree included only the 11 species and sub-species of Testudinidae we used to analyze the relationship between call fundamental frequency and body size, and was based on molecular information reported by Caccone *et al.* (1999*a*,*b*), Meylan (2000), and van der Kuyl *et al.* (2002). In this case, phylogeny was completely resolved (Fig. 2).

Data for different species cannot be considered as independent points in comparative studies because closely related species are more likely to share similar features due to a common ancestor (Felsenstein, 1985; Harvey & Pagel, 1991). However, there is debate about the importance of using comparative methods to control for shared evolutionary history (Ricklefs & Starck, 1996;

Price, 1997; Harvey & Rambaut, 1998), and it has been suggested that species-level analyses may, in some cases, be an appropriate method for analysis of comparative data (Ricklefs & Stark, 1996; Losos, 1999); in fact, differences between results using raw species data and phylogenetic methods may provide some biological insight (Price, 1997). Therefore, we analyzed our data using both the raw species values and comparative methods that control for phylogeny (statistically independent contrasts).

To produce data that were phylogenetically independent under a specific evolutionary model, we calculated standardized linear contrasts (Felsenstein, 1985; Harvey & Pagel, 1991) as implemented by the computer package Comparative Analysis of Independent Contrasts (CAIC, Purvis & Rambaut, 1995). The CAIC program produces linear contrasts that are standardized differences in traits at evolutionarily independent nodes in the phylogeny (Purvis & Rambaut, 1995). Since we have no information about branch lengths, contrasts were standardized assuming that lengths of branches in the phylogeny were either proportional to the number of taxa in each clade (Graphen, 1989), which is similar to a graduate model of evolution, or equal in length, which represents a punctuated model of evolution. In order to verify if branch lengths were appropriate, we computed Pearson correlation coefficients between the absolute value of each standardized independent contrast and its standard deviation (Garland *et al.* 1992). No significant relationships were found for both equal and proportional branch length (all r_p coefficients < 0.28), indicating that contrasts were adequately standardized. Our results were similar with graduated and punctuated branch lengths, so for simplicity we present just the results from the analysis using equal branch lengths.

For both raw species and independent contrasts analyses we used a linear regression model (LRM) to investigate whether variance in fundamental frequency among species was accounted for by body size. Fundamental frequency and carapace length values were $\log_{10}$-transformed. Regression for phylogenetic analysis was forced through the origin, because the mean value of independent contrasts is expected to be zero under the null hypothesis (Harvey & Pagel, 1991).

RESULTS

OCCURRENCE AND EVOLUTION OF MOUNTING-CALLS

Courtship behaviour has been described for 101 chelonian species (Table 2). Among them, mounting-calls were reported for 35 species belonging to the families Testudinidae (n=29, 80.5% of species with described courtship), Trionychidae (n=3, 60% of species with described courtship), Emydidae (n=2, 7.4% of species with described courtship) and Bataguridae (n=1, 8.3% of species with described courtship). To our knowledge, none of the species belonging to other families has been reported to produce mounting-calls. However, we found
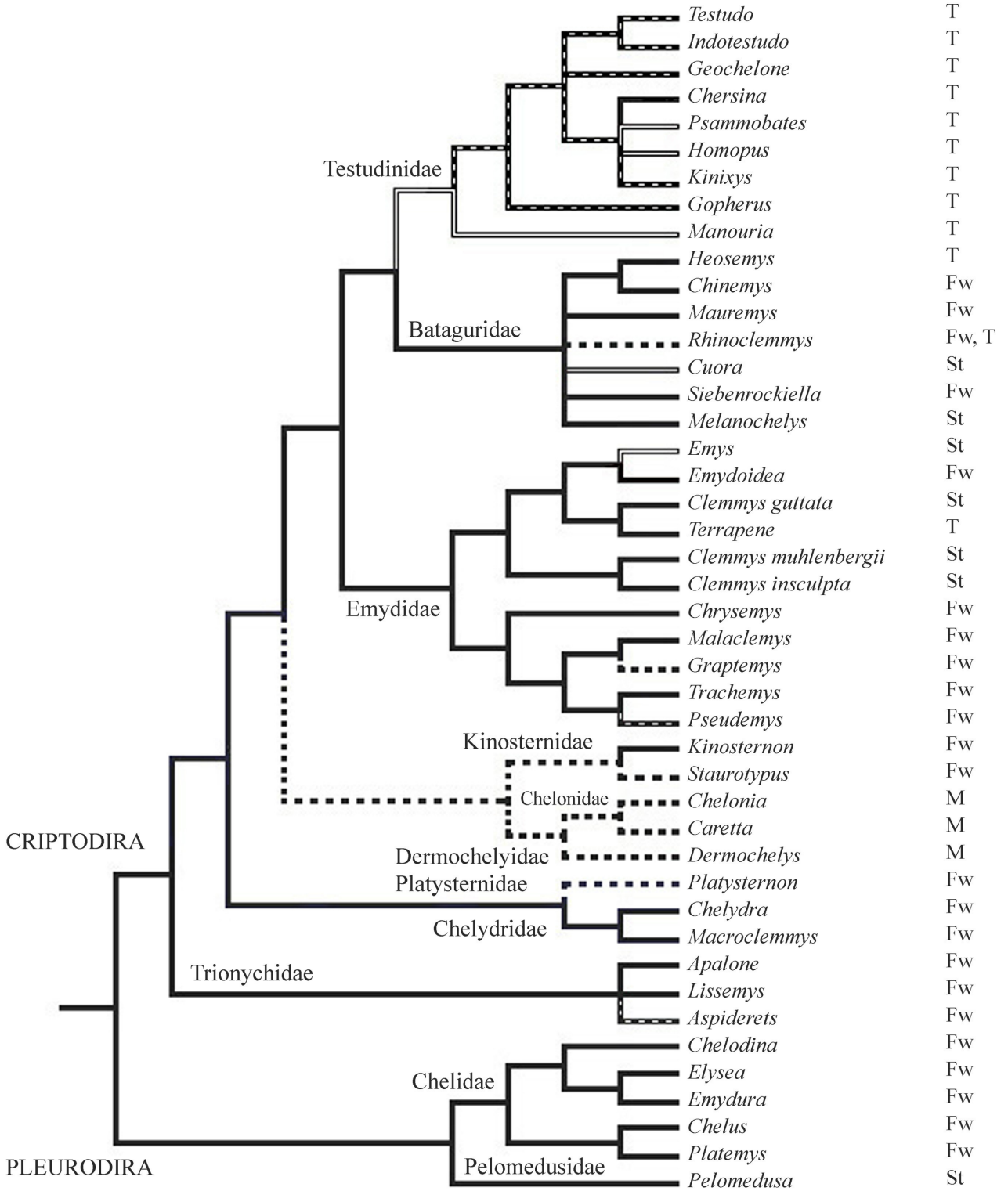
FIG. 1. Phylogenetic tree for chelonian genera (see Methods) whose courtship has been described. Black lines: genera that do not vocalize; white lines: genera including species vocalizing during mounting; black and white lines: genera including species vocalizing both during and outside mounting; dotted lines: genera including species vocalizing outside mounting. Single species are reported for polyphiletic genera. Main habitats are indicated on the right column: T, terrestrial; St, semi-terrestrial; Fw, fresh-water; M, marine.
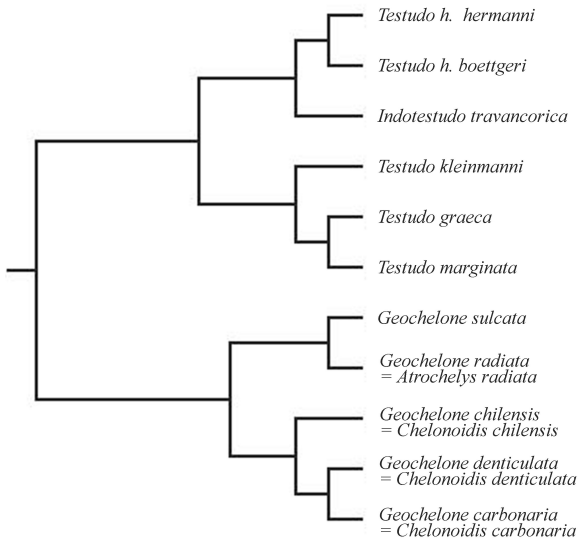
FIG. 2. Phylogenetic tree for the 11 species and sub-species of Testudinidae (see Methods) used to study relationship between body size and call fundamental frequency.

that other call types are used by some species of Cheloniidae Oppel, 1811, Dermochelyidae Fitzinger, 1843, Platysternidae Gray, 1869, Emydidae and Bataguridae outside courtship and mounting, for example during combats or when disturbed (Table 3); these kinds of vocalizations are emitted by both sexes in several species, and also by juveniles of *Geochelone carbonaria* = *Chelonoidis carbonaria* (Spix, 1824) during foraging. Considering that courtship behaviour has been described, at least roughly, for only 41% of species, the ability to produce vocalisations should probably be more widespread in chelonians, and it is definitely not limited to sexual contexts.

The phylogenetic tree of chelonian genera we constructed (Fig. 1) indicated that calls associated with mounting were apparently absent in all Pleurodiran genera, appeared firstly in the ancestral Cryptodiran group of Trionychidae, were maintained in some Emydidae and Bataguridae genera, but were present in all recent Testudinidae, where mounting-calls appeared from the root of the family evolutionary tree (genus *Manouria* Gray, 1852), and was then inherited by the other genera. Therefore, the lack of mounting-calls in the genus *Chersina* Gray, 1831 may be considered a secondary loss.

It is worth noting that, apart from the three fresh-water species of Trionychidae, all the other vocal species are terrestrial or semi-terrestrial, while none of the marine species had been reported to vocalise during courtship and mounting. In fact most marine and fresh-water species mate underwater. Therefore, mounting-calls were significantly related to terrestrial habitats ($\chi^2_1 = 33.7$, $P<0.001$).

TABLE 2. Number and occurrence of chelonian species vocalizing during mount.

Family	Habitat	No. species	No. species with described courtship	% species that vocalize among those with described courtship
Chelidae Gray, 1825	fresh water	36	8	
Pelomedusidae Cope, 1868	fresh water	22	0	
	semi-terrestrial	1	1	
Carettochelyidae Boulenger, 1887	fresh water	1	0	
Trionychidae Fitzinger, 1826	fresh water	21	5	60.0
	unknown	1	0	
Chelydridae Gray, 1831	fresh water	2	2	
Platysternidae Gray, 1869	fresh water	1	0	
Cheloniidae Oppel, 1811	marine	6	2	
Dermochelyidae Fitzinger, 1843	marine	1	0	
Kinosternidae Agassiz, 1857	fresh water	22	8	
Dermatemydidae Gray, 1870	fresh water	1	0	
Emydidae Rafinesque, 1815	fresh water	28	21	4.8
	semi-terrestrial	4	3	33.3
	terrestrial	3	3	
Bataguridae = Geoemydidae Theobald, 1868	fresh water	37	7	
	semi-terrestrial	6	2	50.0
	terrestrial	7	3	
	unknown	6	0	
Testudinidae Batsch, 1788	terrestrial	40	36	80.5
Total		246	101	34.6

TABLE 3. Description, emitting sex and context of vocalizations uttered by turtles and tortoises (M: male, F: female, J: juvenile).

Species	Vocalization	Sex	Context	References
Dermochelys coriacea (Vandelli, 1761)	wail/groans/roar/bellow	M	when attacked	Carr 1952
	roars	M	diving	Kumpf 1964
	?	F	nesting	Mrosovsky 1972
"Sea turtles"	"mercy cry"/roar/grunt	?	?	Carr 1952
Aspideretes hurum (Gray, 1831)	?	M	courtship	Flower 1899
Aspideretes nigricans (Anderson, 1875)	hiss	?	?	Annandale (in Ernst & Barbour 1989)
Aspideretes gangeticus (Cuvier, 1824)	low, hoarse cackle	?	courtship?	Günther 1864
Staurotypus triporcatus (Wiegmann, 1828)	2 voices	?	?	Cope 1865
Platysternon megacephalum Gray, 1831	squeal	J	when disturbed	Campbell & Evans 1972
Rhinoclemmys punctularia (Daudin, 1801)	piping	?	when killed	Cope 1865
Cuoraflavomarginata= *Cistoclemmys flavomarginata* Gray, 1863	lip-smacking/hiss	M	courtship	Connor & Wheeler 1998
Emys orbicularis (Linnaeus, 1758)	low piping note	M	mounting	M. Zuffi, M. Lebboroni, pers. com.
Pseudemys floridiana (Le Conte, 1830)	grunt	?	courtship?	Neil 1950
Graptemys Agassiz, 1857 sp.	?	?	?	cited in Campbell & Evans 1972
Kinixys belliana Gray, 1831	wheezing honks	M/F	combat/ mounting	Morris 1994
Kinixys erosa (Schweigger, 1812)	hissing squeak	M	mounting	Ernst & Barbour 1989
Kinixys homeana Bell, 1827	moaning hiss	M	mounting	Kirkpatrick 1998
Kinixys natalensis Hewitt, 1935	moaning hiss	M	mounting	Kirkpatrick 1998
Homopus signatus (Gmelin, 1789)	squeaking/loud squeak	M	courtship/ mounting	Palmer 1994
Psammobates oculifera = *Psammobates oculiferus* (Kuhl, 1820)	grunting cough	M	mounting	Ernst & Barbour 1989
Manouria emys (Schlegel & Müller, 1840)	moan	M/F	courtship/ mounting	McKeown *et al.* 1990
Indotestudo elongata (Blyth, 1853)	harsh raspy sound	M/F	combat/ mounting	McCormick 1992, C. Tabaka, pers.com.
Indotestudo forstenii (Schlegel & Müller, 1840)	?	M/F	combat/ mounting	C. Tabaka, pers. com.
Indotestudo travancorica (Boulenger, 1907)	pulses	M	nocturnal/	Auffenberg
	grunt	M	chorus	(in Campbell & Evans 1972)
			mounting	Auffenberg 1964
Geochelone elegans (Schoepff, 1794)	grunt	M	mounting	Ernst & Barbour 1989
Geochelone platynota (Blyth, 1863)	grunt-like bellow	M	mounting	www.startortoise.com
Geochelone pardalis (Bell, 1828)	grunt-like bellow	M	mounting	Ernst & Barbour 1989
Geochelone sulcata (Miller, 1779)	grunt or ducklike quack	M	mounting	Grubb 1971

TABLE 3. continued...

Geochelone nigra =				
Chelonoidis nigra (Quoy & Gaimard, 1824)	roar/bellow	M	mounting	De Sola 1930, Evans 1949, Van Denburgh 1914
Geochelone gigantea =				
Dipsochelys elephantina (Duméril & Bibron, 1835)	groan/bellow	M	mounting	Ernst & Barbour 1989
Geochelone radiata =				
Astrochelys radiata (Shaw, 1802)	grunt	M	mounting	Ernst & Barbour 1989
Geochelone yniphora =				
Astrochelys yniphora (Vaillant, 1885)	?	M	mounting	Ernst & Barbour 1989
Geochelone carbonaria =				
Chelonoidis carbonaria (Spix, 1824)	cluck	M	mounting,	Auffenberg 1965,
	cluck or chirp	J	foraging	Campbell & Evans 1967, Campbell 1967
Geochelone denticulate =				
Chelonoidis denticulata (Linnaeus, 1766)	cluck	M	mounting	Snedigar & Rokosky 1950, Auffenberg 1965
Geochelone chilensis =				
Chelonoidis chilensis (Gray, 1870)	grunt or ducklike quack	M	mounting	present paper
Testudo graeca Linnaeus, 1758	whistlelike noise	M/F	combat/ mounting	Ernst & Barbour 1989 present paper
Testudo hermanni Gmelin, 1789	squeaklike grunt	M/F	combat/ mounting	Ernst & Barbour 1989 present paper
Testudo horsfieldiii =				
Agrionemys horsfieldii (Gray, 1844)	cluck/squeak	M	mounting	Cohen 1994
Testudo kleinmanni Lortet, 1883	interrupted rattling	M	mounting	Hoofien 1971
Testudo marginata Schoepff, 1793	guttural utterance	M/F	combat/ mounting	Hine 1982, present paper
Gopherus agassizii (Cooper, 1863)	grunt/moan	M	exploration/ mounting	Ernst & Barbour 1989, Campbell & Evans 1967
Gopherus berlandieri (Agassiz, 1857)	?	M	mounting	Hoseholder (in Weaver 1970)
Gopherus polyphemus (Daudin, 1801)	rasping all/mew/ piteous cry	M	?	Carr 1952, Hallinan 1923

CALL FEATURES AND RELATIONSHIP WITH BODY SIZE

In the species we recorded, tortoises emitted a long sequence of simple calls with regular intervals between them during mount (see Table 1 for details). In both *Testudo* Linnaeus, 1758 (Fig. 3) and *Indotestudo* Lindholm, 1929 genera, the calls sounded like whimpers or bellows, and were highly stereotyped within each individual. Calls were highly modulated in frequency and showed a clear harmonic structure: in *T. marginata* up to 13 harmonics could be observed. The call fundamental frequency in these two genera ranged from 296 Hz of *T. marginata* to 602 Hz of *T. h. hermanni*.

In the genus *Geochelone* Fitzinger, 1835 and *Chelonoidis* Fitzinger, 1835 vocalisations consisted in a series of short "clucks" repeated at regular intervals (Fig. 3). Fundamental frequency in this genus ranged from 110 Hz of *C. carbonaria* Fitzinger, 1835 to 230 Hz

of *G. denticulata* = *Chelonoidis denticulata* (Linnaeus, 1766).

The LRM using raw species data showed that call fundamental frequency was negatively related to carapace length ($F_{1,10}$=17.77, P=0.002, β=-0.81, R^2=0.66); after controlling for phylogeny, the negative relationship between fundamental frequency and carapace length still persisted ($F_{1,9}$=6.33, P=0.033, β=-0.44, R^2= 0.41). Thus, larger tortoise species emitted calls at lower frequencies than smaller ones (Fig. 4).

DISCUSSION

Mounting-calls occur in 35% of chelonian species whose courtship behaviour has been described. This figure probably underestimates the real occurrence of mounting calls, due to our limited knowledge of chelonian mating behaviour. Nevertheless, it indicates that
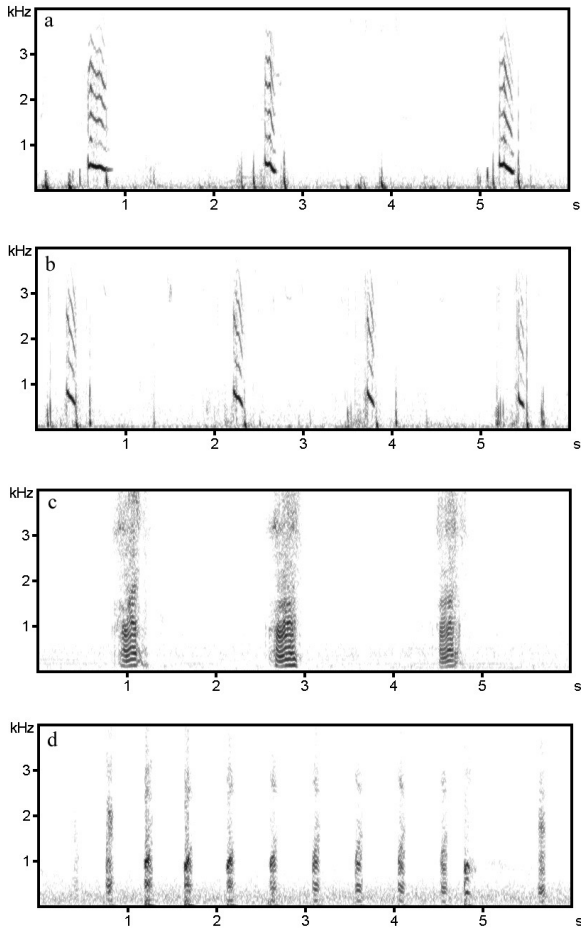
FIG. 3. Sonagrams of mounting-calls emitted by (a) *Testudo hermanni boettgeri*, (b) *Testudo hermanni hermanni*, (c) *Geochelone sulcata* and (d) *Geochelone = Chelonoidis carbonaria*. (FFT: 512, frame: 100%, overlap: 87.5%, window: Flap Top, bandwidth: 59 Hz).
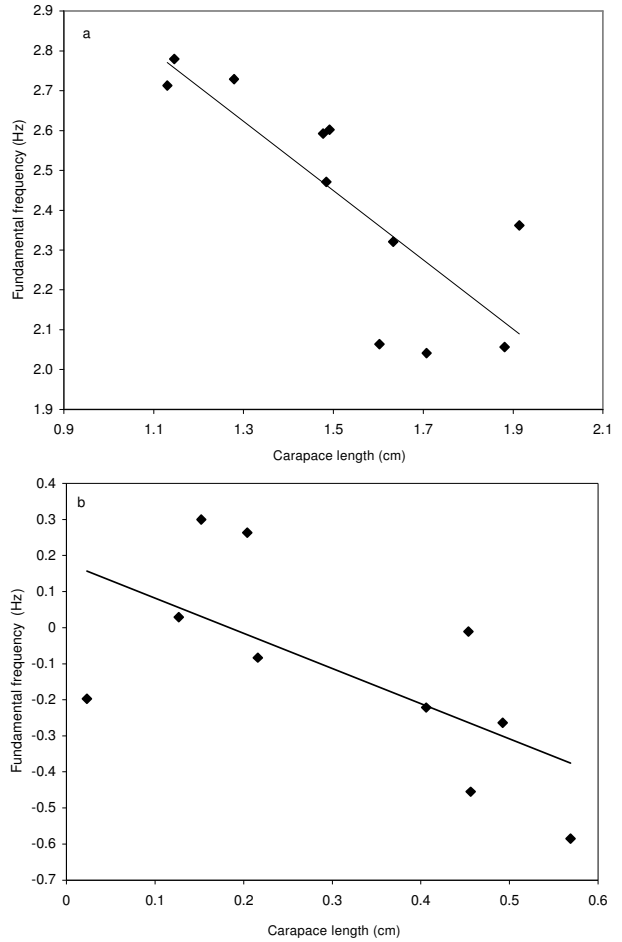
FIG. 4. Relationship between the call fundamental frequency (Hz) and carapace size (cm) for 11 species and subspecies of Testudinidae (a: raw species, b: independent contrasts).

the ability to produce sounds is more widespread in this taxon than previously thought. Moreover, vocalisations are also used outside sexual contexts. The phylogenetic tree we constructed for chelonian genera revealed that mount vocalisations probably evolved in a cryptodirian ancestor, were maintained in some subsequent genera and lost in others, but spread out in all terrestrial Testudinidae. In fact, only four families include species vocalising during mount and most of them (82.8%) belong to Testudinidae. Since vocalizations are maintained throughout the evolutionary history of chelonians, it is likely that mounting calls are functional, considering also that they may attract predators and are costly to produce. For example, in *T. hermanni* both singing rate and call frequency range of mounting males correlate positively with the haematocrit value, and call duration honestly reflects lymphocyte concentration in the peripheral blood (P. Galeotti & R. Sacchi, unpublished data), suggesting that calling requires both a high aerobic capacity (Chappell *et al.* 1997) and good general health condition. The fact that most Testudinidae species vocalise during mating therefore suggests that mounting-calls provide receivers with some useful in-

formation to assess signaller qualities. Signallers, in turn, may gain some selective advantages, ranging from being preferred as sexual partners by females, as documented by their mounting success (Sacchi *et al.*, 2003; Galeotti *et al.*, 2005), to avoiding sexual interferences from other males.

The first line of evidence that mounting-calls function in intraspecific communication comes just from the fact that most chelonian species vocalising during mount are terrestrial or semi-terrestrial, and only three species live in fresh-waters, which is consistent with the lower costs of sound production in terrestrial habitats compared to aquatic ones (an aquatic sender will have to generate sounds with pressure levels 5000 times higher than in air just to provide the same energetic stimulation at receiver's ears, Bradbury & Vehrencamp, 1998). This finding strongly suggests that vocalisations evolved for communicating in terrestrial habitats. If vocalisations were only a by-product of copulatory efforts, they would occur in both terrestrial and aquatic species. However, to the best of our knowledge, no aquatic species produce mounting-call, even in those species where males emerge from water during mount.

The second line of evidence is that vocalisations of tortoise species we recorded showed both frequency and amplitude modulation, and a rich harmonic structure, which are acoustic features commonly used by birds and mammals (Catchpole & Slater, 1995; Manser, 2001) to encode information in intraspecific communication, and are incompatible with mechanisms of sound production based simply on air flow through respiratory tracts; they rather imply the presence of vibrating structures. For example, in a recent paper on the colubrid *Pituophis melanoleucus*, which emits hisses showing a clear harmonic structure, Young *et al.* (1995) found a specific acoustic device that he described as a 'vocal cord'. The evolution of such a complex structure seems to rule out a non-functional explanation for mounting-calls, at least in tortoises.

A third line of evidence that tortoise calls were not simple respiratory noises was supplied by the relationship between call fundamental frequency and carapace length. In both amphibians and birds, this relationship is largely due to the correlation of body size with the mass of the vibrating device producing sounds (Bowman, 1983; Ryan, 1985; Ryan & Brenovitz, 1985). The existence in tortoise species we recorded of the same size-dependent pattern of frequency further supports the existence of specific sound producing structures very similar to those found in other vertebrates.

The analyses we performed on the larynx morphology of three species of Testudinidae demonstrated the existence of fine anatomical structures not yet described: two blind *diverticula* on the bottom of the cricoid structures in *T. graeca* Linnaeus, 1758 and *T. marginata.* Moreover we found two belts of elastic fibres in the larynx wall, that may vibrate under air flow pressure thus generating sounds (Sacchi *et al.*, 2004).

A final consideration supporting the communicative function of mounting vocalisations in this taxon may be added: in all chelonian species for which a call spectrographic analysis has been carried out, the call fundamental frequency generally fell just within the range of ear sensitivity of these species (Wever & Vernon, 1956*a,b*; Gulick & Zwick, 1966; Patterson, 1966; Campbell & Evans, 1967; Ridgway *et al.* 1969).

On the whole, these findings appear to rule out the possibility that vocalisations in tortoises are simple "noises", involuntarily produced by copulatory movements (Weaver, 1970; Mrosovsky, 1972). Based on our study of *T. marginata* (Sacchi *et al.*, 2003) and *T. hermanni* (Galeotti *et al.*, 2005) we suggest that mount vocalisations are reliable condition-dependent signals conveying honest information about male body size, general health condition and mating success to conspecifics.

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