Courtship behaviour, mating season and male sexual interference in *Salamandrina perspicillata* (Savi, 1821)

Giacomo Bruni¹, Antonio Romano²,*

**Abstract.** The knowledge of reproductive behaviour of *Salamandrina perspicillata*, an endemic Italian salamander, is still fragmented and not exhaustive; and the only detailed observations were made just once in a terrarium. We describe many aspects of the terrestrial courtship behaviour such as male alert posture, substrate marking trail, approach and pursuit, tail undulation and vent swinging, and spermatophore deposition and pick-up. The courting pair follows an ellipsoidal track during this manoeuvre a spermatophore is deposited by the male just in front of the female who will reach the spermatophore as she continues to circle. No body contacts were observed during the courtship. Tail movements play a key role in the communication between sexes as well as between antagonistic males. Male-male combat involves biting as the main deterrent. We found that the mating season in wild populations is in the spring, differing from that reported previously for mating in captivity (winter) or extrapolated from the beginning of sperm storage (autumn). Each of these points is discussed in light of available information on social communication, sexual dimorphism, courtship evolution, and sperm storage.

**Keywords:** courtship behaviour, male-male aggressive behaviour, mating season, *Salamandrina*.

**Introduction**

Despite the strongly supported monophyly of the family Salamandridae (Weisrock et al., 2005), salamandrid courtship is characterised by remarkable diversity among genera in amplexus and sperm transfer modes (see Houck and Arnold, 2003, for an exhaustive review). A major theme in urodelan evolution is the innovation in types of amplexus. According to Houck and Arnold (2003), courtship behaviour in the Salamandridae can be classified by the degree of physical contact between the male and the female into five categories: (i) ventral amplexus; (ii) head to head amplexus; (iii) dorsal amplexus; (iv) restraint of the female and (v) complete absence of amplexus. The comparative study of courtship behaviour helps us to understand the evolution of different reproductive strategies and species-specific sexual behaviour patterns in this family (Salthe, 1967; Titus and Larson, 1995; Steinfartz et al., 2006b). Moreover, Salthe (1967) recognised four basic stages in the courtship of salamanders: the preliminary stage A (i.e., the male approach), the stage B in which may be observed different degree of physical contact between sexes, the stage C (not present in all species) that occurs when the male moves away from the female and she follows him (her attention fixed on male cloaca or tail root) and, finally, the stage D that is the deposition of the spermatophore.

One variant of ventral amplexus, where the courting pair face head-to-head and circle but without the forelimbs interlocked, is characteristic of the Italian endemic genus *Salamandrina* as well as some species of *Tylototriton*, a genus widespread in continental Asia (Houck and Arnold, 2003). In the former genus two species have been recently recognised, i.e., *Salamandrina perspicillata* (Savi, 1821) and *S. terdigitata* (Bonnaterre, 1789) (Mattoccia et al., 2005; Canestrelli et al., 2006; see Romano et al., 2009, for details on the distribution of both species). Salamanders of the genus *Salamandrina* can be briefly described as follows. They are tiny salamanders, deep brown or grey-blackish on the dorsal surfaces of the body and tail. The ventral region is white or whitish, with dark grey to black spots. The undersides of the tail and the feet, and frequently the distal part of the belly,
are bright red. On the head a V-shaped whitish or yellowish spot between the eyes forms a sort of “spectacles”, giving rise to the common name “spectacled salamanders”.

A method for determining sex of adult spectacled salamanders (observing the internal cloacal morphology in vivo) has been available only since 2009 (Romano, Bruni and Paoletti, 2009) and sexes were previously considered indistinguishable by external morphology (e.g., Zuffi, 1999; Angelini et al., 2007). The adults are semi-terrestrial and only the females go to the water, during the reproductive season, to spawn their eggs (Lanza, 1983).

Salamandrina courting is terrestrial and it was observed in captivity, in December, by Strötgen (1927). Recently, Houck and Arnold (2003) provided some first-hand observations of courtship behaviour in the field that agree substantially with those reported by Strötgen (1927). However, the former paper is considered lacking in detail (see Salthe, 1967; Titus and Larson, 1995) and both contributions are considered not fully validated under natural conditions by other authors who have carried out research on courtship patterns and phylogeny of the Salamandridae (Utzeri et al., 2005; Angelini, pers. comm. in Steinfartz et al., 2006b). In particular, Steinfartz et al. (2006b), who used molecular phylogeny to map the evolution of life history and courtship traits in salamandrids, considered the data on Salamandrina courtship behaviour unresolved (especially in regard to whether or not physical contact is involved in the courtship) and thus problematic for their study.

The information on courtship behaviour in Salamandrina is, therefore, still fragmentary and incomplete. In recent years, some aspects of the natural history, distribution, biology, biogeography, ecology and behaviour of Salamandrina, and of S. perspicillata in particular, have been elucidated (e.g., Della Rocca, Vignoli and Bologna, 2005; Angelini, Antonelli and Utzeri, 2008; Romano, Forcina and Barbanera, 2008; Romano et al., 2009; Romano and Ficetola, 2010). However, knowledge is still very fragmented with regard to many aspects of their reproductive behaviour (in particular courtship stages C and D; see Salthe, 1967, and mating season in the wild). A more thorough description on courtship behaviour of Salamandrina was needed, therefore, to clarify the courtship display and mating season of these Italian endemic salamanders in the wild. In the present note, we report first-hand observation of courtship, modes of sperm transfer, mating season and male-male competition in Salamandrina perspicillata. As in other papers reporting analogous behaviour, the descriptions are mainly and necessarily of a qualitative nature (e.g., Sparreboom, Steinfartz and Schultschik, 2000; Sparreboom, Xie and Fei, 2001; Hidalgo-Vila, Pérez-Santigosa and Díaz-Paniagua, 2002).

**Materials and methods**

The studied population of Salamandrina perspicillata spawns in a slow-running stream (near Sambuca Pistoiese, Tuscany, Central Italy; datum WGS84, coordinates: 44°06’28”N, 10°58’14”E; altitude: 485 m a.s.l.) in a deciduous forest dominated by the Common Beech, Fagus sylvatica, and by the Hornbeam, Carpinus orientalis. The microclimate surrounding the stream is very humid and salamanders are often seen walking on the leaf litter during the day as well as at night. Field observations on the behaviour of S. perspicillata were carried out mainly in 2008 and discontinuously up to May 2010. On 2008 we described the season (i.e., the spring, see table 1) and the hours when salamanders were highly active (6:30-10:00 a.m.) and, in the 2009 and 2010, we focused our field researches mainly in this season and in this time interval (at least for two consecutive hours). Meteorological parameters were not measured on the study site, however we reported the data recorded by a weather stations at 500 m a.s.l. and 4.8 km from the study site (near Porretta Terme, Emilia Romagna region; table 1). When we observed salamanders (both interacting and not interacting), we waited for the end of their particular behaviour and, then, we collected and handled the salamanders to determine their sex on the basis of cloacal morphology (following the method described by Romano, Bruni and Paoletti, 2009). We did not mark individually the salamanders and, thus, among different sampling dates, they were not recognisable. During field research we used a small LED torch just in the nocturnal sampling (between the complete sunset and the dawn). We describe behaviour patterns...
Table 1. Behaviour patterns observed in surveys of a population of *Salamandrina perspicillata* from Tuscany, at 485 m a.s.l., by date. Observations were made between 6:30 and 10:00. The presence of salamanders and of any given behaviour were scored as observed (+) or unobserved (−) when no numeric data were recorded or, alternatively, the number of the observations was reported. MM = males; FF = females. Meteorological parameter were recorded by a weather stations at 500 m a.s.l. and far 4.8 km from the study site.

<table>
<thead>
<tr>
<th>Date</th>
<th>Non-interacting salamanders</th>
<th>Male watchful behaviour</th>
<th>Male-male interactions</th>
<th>Incomplete mating</th>
<th>Complete mating</th>
<th>Mean temperature</th>
<th>Minimum temperature</th>
<th>Maximum temperature</th>
<th>Precipitation</th>
<th>Relative humidity</th>
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</thead>
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<tr>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>6.2°C</td>
<td>4°C</td>
<td>7.3°C</td>
<td>1 mm</td>
<td>95%</td>
</tr>
<tr>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>7.4°C</td>
<td>3°C</td>
<td>13°C</td>
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<td>60%</td>
</tr>
<tr>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>7.3°C</td>
<td>3.4°C</td>
<td>13°C</td>
<td>−</td>
<td>49%</td>
</tr>
<tr>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>17.1°C</td>
<td>−</td>
<td>62%</td>
</tr>
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<td>+</td>
<td>8</td>
<td>−</td>
<td>−</td>
<td>16°C</td>
<td>10°C</td>
<td>22°C</td>
<td>−</td>
<td>61%</td>
</tr>
<tr>
<td>13 April 2008</td>
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<td>−</td>
<td>1</td>
<td>−</td>
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<td>6.7°C</td>
<td>16.7°C</td>
<td>1 mm</td>
<td>76%</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>4.9°C</td>
<td>6.8°C</td>
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<td>62%</td>
</tr>
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<td>3</td>
<td>4</td>
<td>−</td>
<td>16°C</td>
<td>9.6°C</td>
<td>22.5°C</td>
<td>1 mm</td>
<td>64%</td>
</tr>
<tr>
<td>2009</td>
<td>−</td>
<td>+</td>
<td>5</td>
<td>−</td>
<td>−</td>
<td>13°C</td>
<td>7°C</td>
<td>20°C</td>
<td>−</td>
<td>71%</td>
</tr>
<tr>
<td>2010</td>
<td>−</td>
<td>+</td>
<td>3</td>
<td>−</td>
<td>−</td>
<td>17°C</td>
<td>13°C</td>
<td>23°C</td>
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<td>76%</td>
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<tr>
<td>26 April 2008</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>6</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>62%</td>
</tr>
<tr>
<td>2010</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>6</td>
<td>−</td>
<td>7.5°C</td>
<td>20°C</td>
<td>−</td>
<td>−</td>
<td>62%</td>
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<td>+</td>
<td>+</td>
<td>2</td>
<td>4</td>
<td>−</td>
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</tr>
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<td>2</td>
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<td>−</td>
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<td>8.9°C</td>
<td>22.6°C</td>
<td>−</td>
<td>46%</td>
</tr>
<tr>
<td>29 April 2008</td>
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<td>+</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>13.5°C</td>
<td>10°C</td>
<td>17.1°C</td>
<td>4 mm</td>
<td>74%</td>
</tr>
<tr>
<td>1 May 2008</td>
<td>+</td>
<td>+</td>
<td>3</td>
<td>5</td>
<td>−</td>
<td>6.8°C</td>
<td>10°C</td>
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<td>−</td>
<td>59%</td>
</tr>
<tr>
<td>2009</td>
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<td>+</td>
<td>1</td>
<td>5</td>
<td>−</td>
<td>16°C</td>
<td>11°C</td>
<td>22°C</td>
<td>1 mm</td>
<td>65%</td>
</tr>
<tr>
<td>3 May 2008</td>
<td>−</td>
<td>+</td>
<td>1</td>
<td>2</td>
<td>−</td>
<td>17.6°C</td>
<td>10.6°C</td>
<td>24.1°C</td>
<td>−</td>
<td>53%</td>
</tr>
<tr>
<td>4 May 2008</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td>5</td>
<td>−</td>
<td>16.8°C</td>
<td>10.8°C</td>
<td>23.3°C</td>
<td>−</td>
<td>58%</td>
</tr>
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<td>11 May 2008</td>
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<td>2</td>
<td>−</td>
<td>−</td>
<td>16.7°C</td>
<td>9.6°C</td>
<td>23.5°C</td>
<td>−</td>
<td>52%</td>
</tr>
<tr>
<td>17 May 2010</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>18°C</td>
<td>13°C</td>
<td>22°C</td>
<td>−</td>
<td>52%</td>
</tr>
<tr>
<td>18 May 2010</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>18°C</td>
<td>10°C</td>
<td>24°C</td>
<td>−</td>
<td>48%</td>
</tr>
<tr>
<td>14 June 2008</td>
<td>24 MM, 3 FF</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>14°C</td>
<td>12°C</td>
<td>18°C</td>
<td>46 mm</td>
<td>81%</td>
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<tr>
<td>15 July 2008</td>
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<td>−</td>
<td>−</td>
<td>22°C</td>
<td>16°C</td>
<td>29°C</td>
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<td>51%</td>
</tr>
<tr>
<td>6 September 2008</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>27°C</td>
<td>20°C</td>
<td>34°C</td>
<td>−</td>
<td>51%</td>
</tr>
<tr>
<td>6 October 2008</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>16°C</td>
<td>8°C</td>
<td>22°C</td>
<td>−</td>
<td>54%</td>
</tr>
<tr>
<td>18 October 2008</td>
<td>2 MM</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>14°C</td>
<td>11°C</td>
<td>17°C</td>
<td>9 mm</td>
<td>83%</td>
</tr>
<tr>
<td>19 October 2008</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>15°C</td>
<td>12°C</td>
<td>18°C</td>
<td>−</td>
<td>71%</td>
</tr>
<tr>
<td>4 November 2008</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>15°C</td>
<td>13°C</td>
<td>16°C</td>
<td>13 mm</td>
<td>94%</td>
</tr>
<tr>
<td>15 November 2008</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>10°C</td>
<td>7°C</td>
<td>14°C</td>
<td>−</td>
<td>86%</td>
</tr>
<tr>
<td>10 December 2008</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>3°C</td>
<td>1°C</td>
<td>7°C</td>
<td>1 mm</td>
<td>95%</td>
</tr>
</tbody>
</table>
of both sexes. To provide a comparable description of behaviour patterns between sexes, we used a numeric progressive code and the same number corresponds with the overlap between male and female behaviour. We provide crude data on the number of interactions between salamanders as observed on the sampling dates. Some behaviours were recorded as video files with a low resolution video camera. We combined our observation with that unpublished of Stevan J. Arnold which were collected, on 1983 in the field (near Cardoso, Stazzema, Tuscany), and in 1984 in the laboratory.

Results

All courtship interactions and other correlated behaviours we observed were terrestrial. We observed various behaviour patterns (described below), including incomplete courtship sequences and a complete mating. Incomplete sequences were observed a number of times and a complete mating was observed on 29 April 2008 at 7:30 a.m. Furthermore, on 13 April 2008, upon opening the cloacas of two salamanders to sex them (following the procedure set forth in Romano, Bruni and Paoletti, 2009), both were found to be females and two spermatophores were detected, presumably having been picked up only a short time prior. On this date the first spawned eggs were also recorded. Our field observations are recounted in table 1. The following ethogram lists behaviour patterns observed during the surveys.

Behaviour patterns shown by the males

1. Alert posture (observed several times). The male exhibits a watchful behaviour. With his head raised, he climbs onto a small heap of dead leaves, rocks or other supports that may provide a better view of the surroundings (fig. 1a, b). To raise himself up, the male often leans his forelimbs on stems and leaves. He can remain motionless for a long periods (15-30 minutes at least, S.J. Arnold’s field observation, pers. comm.). Sometimes the male exhibits “stand-up” behaviour, standing up on the hind limbs and using the tail for support (fig. 1c).

2. Substrate marking trail (observed a few times). The male slightly raises the cloaca and

Figure 1. Alert posture and “stand-up” behaviour of a male of *Salamandrina perspicillata*. (a) Watchful behaviour is indicated by the raised head sustained by the stretched front limbs. (b) Often the male climbs on small heap of dead leaves, rocks or other supports. (c) The male stands up on his hind limbs and is supported by his tail.
the basal part of the tail and excretes a slimy string of transparent mucous.

3. Approach, pursuit and nudge (observed several times). When a female is in the vicinity of a male and within his visual field, then the male quickly approaches the female. The male follows the female, walking or running after her or parallel to her body. While the pair walks, the male tries to bar the female’s way, positioning himself in front of her. The male keeps his head raised. Sometimes his snout makes one or more brief contacts with the lateral part of the female’s body or head or with the basal part of the female’s tail. In the excitement of the pursuit, the male can walk right over the female and can execute a turn while he is doing that. Only once did we observe tail undulation while the male approached the female (see point 5 below).

4. Continuation or interruption of the courtship (observed several times). If the female is unresponsive (see female behaviour), the male stops the courting. If the female is responsive and stops moving, then the courtship continues.

5. Tail undulation and vent swinging (observed several times). If the female stops moving (is a responsive female), then the male begins to move his tail in a horizontal plane (fig. 2a). The male may already be positioned in front of her, or may be positioned at her flank, or sometimes with forelimbs leaned on the back of the female. In the latter two cases, the male slowly moves to position himself in front of the female. When the male is in front of the female, he puts his snout near the pelvis of the female and the couple is positioned in an elliptical pattern. Two sub-stages within the tail-undulation stage in *S. perspicillata* may be recognised. The first is a slow undulation and the second is a quick undulation of the tail. The slow undulation consists of the sinuous tail movements which begin at the tail tip and run along the tail towards the base where the undulation is more pronounced. The male also moves his pelvis slightly, mainly from side-to-side in a horizontal plane. After this sub-stage, if the female does not move away (i.e., she continues to be responsive), the male begins the quick undulation, a much more vigorous undulatory movement of the tail, which is synchronous with a slight lifting of his pelvis and cloaca from the ground.

6. Circling, run and shiver (observed once, fig. 2b, c). After the quick undulation the male (as well as the female) begins to walk, positioning himself to assume a head-to-head posture and beginning to circle anti-clockwise. After some circling along an ellipsoidal route, the male quickly moves his body, and his pelvis in particular, in a side-to-side shivering motion. The male carefully adjusts his position in relation to that of the female, so that the bodies of the courting pair are separated by a distance of 2-4 cm.

7. Spermatophore deposition (observed once, fig. 2d). During the circling and shivering, the male stops himself suddenly and slightly raises his undulating tail. He deposits a spermatophore approximately in front of the female who will reach the spermatophore as she continues to circle. The size of the spermatophore is 4 mm high (S.J. Arnold, pers. comm.). After spermatophore deposition the pair continues to circle and then they stop again when the female reaches the spermatophore point (where she is stopped by the male that stands still and undulates his pelvis). The male continues to move his tail sinuously.

8. Pair separation (observed once). By the time the spermatophore has been picked up, the pair is oriented approximately parallel but in opposite directions (as in a very elongate ellipse), with the snout of the male near the female’s cloaca and vice versa (fig. 2e). The male rests briefly in the same place and finally he moves away from the courtship behaviour area.

9. Male sexual interference (observed several times). An ongoing courtship may be interrupted, at any stage, by an interfering male moving towards a courting pair. In such cases, the male involved in the courtship (courting
Figure 2. Diagrammatic representation of reproductive behaviour in *Salamandrina perspicillata*. The circle shows the path of the pair’s cloacae. Small curved lines indicate movements of a given part of the body (tail undulation, vent swinging). The female (in grey) is slightly larger than the male (in black). The grey triangle represents the spermatophore. (a) The male follows the female, walking or running after her or parallel to her body and, if the female is responsive, the male begins to move his tail in a horizontal plane. (b) The female brings her head towards the male pelvis and the pair begin to circle. (c) The two courting salamanders are positioned in an ellipsis and circle; both sexes exhibit tail undulation. (d) The male slightly raises his undulating tail and, pressing his cloaca on the substrate, he deposits a spermatophore approximately in front of the female. (e) The female turns another half circle, moving her pelvic region until she is over the spermatophore and, then, she lowering her cloaca. She stretches the tail and performs a vent swinging both side-to-side and back and forth (indicated by the arrows). The dorsal sperm-filled cap of the spermatophore lodges within the female’s cloaca.

male) approaches the interfering male quickly while moving his body jerkily. The courting male walks with a raised head that is further raised and lowered suddenly and repeatedly. The courting male moves the tail aggressively with wide and fast movements sideward. Sometimes the courting male tries to overtake the interfering male and he may briefly pursue the intruder. If the interfering male does not show submissive behaviour (moving away from the area of the courtship), the courting male exhibits a more aggressive behaviour, i.e., bites on several parts of the body of the intruder (we observed actual acts of biting on flanks, tails, feet; see fig. 3) but more frequently on the intruder’s head. The bites are long lasting and a biting courting male can be transported by the escaping interfering male for a considerable distance (we observed up to 50 cm). Once we observed two interfering males that interrupted an on-going courtship. The courting male approached the two interfering males and reached them at about 20 cm from the courting area. The courting male engaged in a violent defence versus the two intruders, with fast bites and rapid movements of both the body and the tail. Both
the interfering males escaped; the courting male returned near the female and the pair restarted mutual tail undulation. However, for unknown reasons, unexpectedly, the female moved away.

**Behaviour patterns shown by the females**

1. **Entry in the male visual field** (observed several times). While a female is walking, she enters into the male’s visual field.

4. **Female responsive or unresponsive behaviour** (observed several times). An unresponsive female may show lack of interest toward the male by continuing to walk or she may decline more robustly by exhibiting fast tail tip undulation for a brief period. Alternatively the female accepts the courtship by stopping her movement.

5. **Tail undulation** (observed several times, fig. 2a). The female, if responsive, stands still and allows the male to position himself in front of her. The female’s snout is near the male’s flank in proximity to the male’s cloaca while the male begins to move his tail. An unresponsive female can move away from the male during the stage of the male’s tail slow undulation. If she doesn’t move away and the male passes into the tail quick undulation stage, then the female moves her tail tip slowly and sinuously (a worm-like movement called ‘wiggle tail bent’ in some species of newts; see Arntzen and Sparreboom, 1989).

6. **Circling and snout-flank contact** (observed once, fig. 2b, c). Male and female are positioned in opposite directions. The female slightly touches (or skims) with the snout the male’s flank, at which point the male suddenly begins circling. The female we observed pursues the male in circling, following a circular track (see male behaviour).

7. **Spermatophore pick-up** (observed once, fig. 2d). The female turns another half circle, moving her pelvic region until it is over the spermatophore and then lowering her pelvis. The female stretches her tail and stop the tail undulation. The female performs an evident vent swinging both side-to-side and back and forth which may aid in spermatophore pickup (see arrows in fig. 2d). The dorsal sperm-filled cap of the spermatophore lodges within the female’s cloaca. Just after the spermatophore pick-up the female walks away.

**Discussion**

In the study site, *Salamandrina perspicillata* is active mainly from Mach to June, in particular during rainy days (see table 1). In the courtship of *Salamandrina perspicillata* few stages are clearly separated but a given stage may flow into another one without a clear transition. Male alert posture, pursuit of the female, tail undulation and vent-swinging and circling may be considered the basic forms of the courtship display through which the two salamanders communicate by visual and, very probably, by chemical stimuli as they attempt to find the right reciprocal position to permit spermatophore transfer. We analysed separately the points of courtship

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**Figure 3.** Male-male aggressive behaviour in *Salamandrina perspicillata* is generally expressed by bites on the tail (a), on the feet (b), on the flank and, frequently, on the head of the competitor.
behaviour that we think are of particular interest either because they are the most fragmentary and incomplete or because they are completely unknown for Salamandrina. For each point we provide an essential survey of the available literature and a related critical discussion in light of our observations.

Courtship behaviour

Salamandrina perspicillata courtship behaviour has been characterised as a courtship circle without the forelimbs interlocked (Strötgen, 1927) in a head-to-head and pin-wheel fashion (Houck and Arnold, 2003). We chose not to discuss the work of Bruno (1976) due to a lack of reliability of his data (see Lanza, 1988; Zuffi, 1999; Houck and Arnold, 2003; Steinfartz et al., 2006b; Violani and Barbagli, 2006). However, the knowledge of the degree of amplexus has not been considered exhaustive (Salthe, 1967; Steinfartz et al., 2006b). Our observations on sexual behaviour largely agree with those reported by Strötgen (1927) and by Houck and Arnold (2003) and with the additional and unpublished data of S.J. Arnold (in litteris). However we also found some slight differences and we have provided additional data on both courtship behaviour and on the mating season, as observed in the wild.

During the sperm transfer stage of courtship, we and S.J. Arnold (unpublished data) observed the pair circling (fig. 2) with no full physical contact between male and female. In contrast to previous reports, we observed the male and female to be closer than depicted by Houck and Arnold (2003). The courting couple forms a circle (or an ellipsis) with a diameter of approximately 10-13 cm (tail excluded). Each salamander has the snout towards, and relatively close to, the cloaca of the other sex during the entire process of circling (fig. 2) and they carefully keep a certain distance from each other. In our observation, the apparent stimulus to begin the circling is the snout touch (or skim) of the female on the male’s flank (fig. 2b). The deposition of the spermatophore is in front of the female but less than the distance of two body lengths that was previously suggested for Salamandrina, Pleurodeles (aquatic) and Tylototriton by Houck and Arnold (2003). The courtship of S. perspicillata seems to be very similar to the courtship showed by Echinotriton chinhaensis and Tylototriton shanjing, consisting of terrestrial and extensive dance-like circular movements without clapping (in contrast to T. verrucosus, which exhibits aquatic courtship; see Mudrack, 2005). During the complete mating we observed, the male deposited just one spermatophore but S.J. Arnold (pers. comm.) observed, in the field, multiple spermatophore depositions (2-5).

Tail movement (slow and sinuous undulations, quick and sinuous undulations, wiggle tail bent) seems to play a key role in intersexual communication. Different modes of tail undulation are used by the female that may communicate her receptivity or unreceptivity to courtship. Since some species of salamanders are able to obtain information about conspecifics (i.e., sexual identity and reproductive status) through volatile chemical signals (Dantzer and Jaeger, 2007), further studies may test whether, in Salamandrina, tail movements are used to push chemical signals through the air in addition to visual stimuli. We also observed a male, prior to approaching a female, marking the substrate with a slimy string of transparent mucous substance excreted from his cloaca. This behaviour could be interpreted as the release of sexual pheromones although a role for this substance in other social communications (e.g., territorial marking) cannot be ruled out and further research is need to clarify this point.

Many salamander species are most active in dim light or semi-dark conditions, so visual signals often take a form that can be perceived in dim light (Wells, 2007), as that we observed in S. perspicillata, such as changes in body posture and movements of the tail.

Observations of courtships by S.J. Arnold (unpublished data) were made in the field at the end of April, between 19:50 and 21:50.
We observed a high activity pattern during the dawn and the early hours in the morning and the mating was observed at 7:30. Our findings contrast to that reported by Utzeri, Antonelli and Angelini (2004) that observed salamanders to be more active between 18:00 and 24:00 a.m. Differences in daily activity pattern could also be due to the different environments where observations were made, i.e., a very humid and cool habitat with a high cover of trees (our site) and a more dry and open habitat (the study site of Utzeri, Antonelli and Angelini, 2004). On the contrary, our observations agree to that of Utzeri, Antonelli and Angelini (2004) revealing a low activity of salamander on the ground after midnight through dawn.

**Courtship evolution**

Although courtship of urodele species partially evolved in relation to the physical environment in which courtship occurs (Salthe, 1967), however differences and similarities among urodelan species courtships seem to be more strongly correlated with their phylogenetic relationships (see Houck and Arnold, 2003). That means, in turn, that primary selective pressures on mating behaviour may result from sexual selection.

Phylogenetically, “newts” (aquatic or semi-aquatic salamandrids) are separated from the “true salamanders” (semi-terrestrial or completely terrestrial species; Titus and Larson, 1995; Steinfartz et al., 2006b). Although the exact phylogenetic placement of *Salamandrina* remains ambiguous (Titus and Larson, 1995; Veith et al., 1998; Steinfartz et al., 2006b; Weisrock et al., 2006), this genus is unanimously recognised to be the sole extant representative of an ancient lineage of the family Salamandridae, as shown by morphological and molecular analysis (e.g., Thorn, 1969; Titus and Larson, 1995; Steinfartz et al., 2006b). In most of the studies that attempt to reconstruct phylogenetic relationships of salamanders and newts, *Salamandrina* seems to be more or less strictly related to the *Pleurodeles-Tylototriton* group (Titus and Larson, 1995; Steinfartz et al., 2006; Weisrock et al., 2006) and this evidence agrees with the observation of the similarity between *Salamandrina* courtship and that reported in *Pleurodeles* and *Tylototriton* (at least for the *Tylototriton* clades which perform mating on land).

Our observations on sexual behaviour agree with the ancient origin of the genus *Salamandrina*, because *Salamandrina* courtship behaviour shows ancestral characters. The amplexus variant “head to head” without forelimb interlocking (which is directly derived from ventral amplexus, the most ancestral form of amplexus) and the mode of sperm transfer (pinwheel transfer of sperm in front of the female, with male and female facing each other) are both features of extremely ancient origin (Houck and Arnold, 2003).

The splits between the genus *Salamandrina* and other salamandrids and between “true salamanders” and “newts” are probably contemporaneous (about 95 Mya, Steinfartz et al., 2006b). Each species of the basal salamandrid clades (“true salamanders” and “New World newts”) exhibits body contact during courtship, although this has been lost in “modern Eurasian newts” (Houck and Arnold, 2003; Steinfartz et al., 2006b) or secondarily re-evolved in *Europtus* and *Calotriton* (European newts) during the transition from pond-breeding to running-water breeding (Salthe, 1967). The courtship behaviour of *S. perspicillata* is quite unlike that of any other salamander. In particular, the complete absence of physical contact between sexes, excluding the preliminary phases of the courtship (the pursuit), is unknown for other salamandrids that mate on land but reproduce in water (e.g., some “true salamanders” such as *Chioglossa lusitanica*, *Salamandra salamandra* and *S. corsica*). However, molecular phylogenetic perspectives suggest that *Salamandrina* is probably more closely related to the “newts” rather than to the “true salamanders” (Titus and Larson, 1995) and, thus, the absence of body contact could represents a derived loss of amplexus which is a character shared with modern
Eurasian newts. However the hypothesis that the absence of body contact in *S. perspicillata* is a new acquisition (from the salamanders that mate on land) cannot be ruled out. Further research is needed to resolve this issue.

**Mating season and sperm storage**

Sperm storage by females is known to occur in most species of urodeles and one species of anuran (Sever et al., 2001), and it is hypothesized to occur in caecilians (Sever, 2002; Measey et al., 2008). Sperm storage in *Salamandrina* occurs from November to May (Brizzi et al., 1995) making it the longest sperm storage period in Urodela (Houck and Arnold, 2003; see also Sever, 2002; Steinfartz et al., 2006a). Mating in *Salamandrina* was observed in a terrarium, between 2:00 and 3:00 p.m. on December 9 (Strötgen, 1927). Thus a mating season has been deduced (cf. Lanza, 1983; Zuffi, 1999; Sever, 2002) from a single observation in captivity (Strötgen, 1927) and from the beginning of female sperm storage as occurring in the wild population studied by Brizzi et al. (1995). The abundance of sperm in the spermathecae until April and their relative freshness was interpreted as evidence of reduced and slow sperm degradation post-mating (proposed to occur in autumn by Brizzi et al., 1995). However we observed a complete mating at the end of April (table 1), and complete matings were also observed in spring (28-30 April 1983) by S.J. Arnold (unpublished data), in another population of *S. perspicillata* in Tuscany. Consequently at least two mating seasons (autumn and spring) could be considered to occur in *Salamandrina*. However, in our opinion, the autumn could simply be considered the early portion of an extended mating season (as observed for other species, e.g., *Notophthalmus viridescens*; Sever, 2006), and abundance of sperm could depend on multiple matings in different months. Unexpectedly, we did not observe male-female interactions in autumn and winter (see table 1) as we expected, considering the date of mating reported by Strötgen (1927; early December, in captivity). However our research efforts in the field were not homogeneous among different seasons. Consequently, further efforts are needed to verify the span of the mating season in natural populations.

**Watchful behaviour**

During watchful behaviour (fig. 1) the males of *S. perspicillata* display the throat colour and pectoral girdle coloration or sometimes the whole ventral coloration (during the “stand up” behaviour which was reported for the first time, for salamanders of unknown sex, by Utzeri, Antonelli and Angelini, 2005). Ventral and pectoral coloration of *Salamandrina* consist of irregular black, red and whitish patches, with patterning differing among individuals (Vanni et al., 1997), among conspecific populations (Costa et al., 2009) and between species (Angelini et al., 2010). It has also been proposed that this coloration could play a role in inter- (Angelini et al., 2010) and intra-specific communication (Costa et al., 2009) although differences of ventral coloration between the sexes were unknown since the method to distinguish the gender in live salamanders was published only recently (Romano, Bruni and Paoletti, 2009).

We observed watchful behaviour only in males. However, this behaviour was exhibited when no other salamanders were in the visual field (as was also reported for the stand-up behaviour in Utzeri, Antonelli and Angelini, 2005). Watchful behaviour was interrupted suddenly when another salamander entered the visual field and then followed by another type of behaviour (i.e., approach and pursuit). A similar behaviour (salamanders keeping the forepart of the body raised and looking with particular attention all around) was reported also for the completely black salamander *Salamandra lanzai* (Andreone, 1992) and for the fire salamander *Salamandra salamandra* (Joly, 1966). For *Salamandra lanzai* such behaviour was interpreted as a territorial and sexual behaviour (Andreone, 1992), and, for *S. salamandra*, just as a sexual behaviour which is induced, probably, by

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female scent cues that the male finds on the substrate (Joly, 1966). In the males of fire salamander, the watchful behaviour was exhibited when no other salamanders were in the visual field (as in the case of *Salamandrina*) but, more frequently, when a female was in the vicinity of the male (Joly, 1966). Consequently, in our opinion, the hypothesis of the visual communication in *S. perspicillata* using the exhibition of the ventral colour pattern is not completely convincing and requires further investigation. On the basis of our observations, we posit that the exhibition of the ventral colour pattern is not the main reason that salamanders raise themselves but the secondary consequence of this behaviour (with unknown implications) which is adopted, probably, to amplify the visual field, watching from a higher position to more easily detect other conspecifics.

**Male-male interactions**

Aggressive behaviours (fig. 3; bites, attempts to turn a conspecific, quick opening and shutting of the mouth) were reported for pairs of presumed males of *S. perspicillata* (Utzeri, Antonelli and Angelini, 2004).

We confirm that all the behaviours described by Utzeri, Antonelli and Angelini (2004) for only presumed males can be ascribed with certainty to male-male interactions which may be observed either during an interrupted courtship caused by intruders or between males without females present. As is the case for inter-sexual interactions, tail movement also seems to play a key role in social communication between males. The tail is used, in particular, in aggressive advertisements and precedes more aggressive behaviours that involve full body contact. The male tail movements used in the aggressive behaviours are different (more ample, fast, vigorous and less sinuous) than those exhibited in male-female interactions.

Males of *S. perspicillata* have a proportionally wider head than females (Vanni, 1981; Romano, Bruni and Paoletti, 2009). Three alternative or concurrent models of evolution have been proposed to explain such dimorphism: differences in diet between sexes, differences in the reproductive roles and evolutionary advantage for agonistic behaviour in male-male combat (Romano, Bruni and Paoletti, 2009). However, recent data on the diet of *S. perspicillata* showed no differences in diet between sexes (Romano et al., in press). The aggressive interactions reported in this paper corroborate the hypothesis that sexual selection (male-male combat) drives wider heads in males, particularly if receptive females are a limiting resource for males (sex ratio in *Salamandrina* seems to be strongly male-biased; Romano, Bruni and Paoletti, 2009; Vignoli et al., 2010). Indeed, the relationship between head shape and biting (i.e., aggressive behaviour) is intuitive, with head size and shape assumed to be good predictors of bite strength in lizards (e.g., Molina-Borja et al., 1998; Herrel et al., 2001; Adams, 2004), which share with Urodela the overall body architecture (Kupfer, 2007), and in salamanders (Adams, 2004).

**Conclusion**

The observations on the reproductive behaviour of *Salamandrina perspicillata* here reported provide much needed details on the courtship of one of the most interesting genera of the family Salamandridae which occupies a basal phylogenetic position.

Information on the courtship of many salamander species were often obtained in captivity (e.g., Sparreboom, Steinfartz and Schlütschik, 2000; Sparreboom, Xie and Fei, 2001; Hidalgo-Vila, Pérez-Santigosa and Díaz-Paniagua, 2002). Since captivity may influence the behaviour of the tested animals in some unknown way (see Sparreboom and Mouta Faria, 1997), field observations, such as reported here, have an intrinsic additional value because they provide information on the courtship in natural habitat. However our data are far from exhaustive and additional research is need to clarify aspects such as the temporal organisation of the courtship, the number of spermatophores that
are deposited and courtship behaviour in the sister species *Salamandrina terdigitata*.

Mating behaviour may show intraspecific variation in diverse animal taxa including arthropods, fish, amphibians, reptiles, birds and mammals (Lott, 1991; Andersson, 1994; Brock and Verrell, 1994; Taborsky, 1994; Brockmann, 2001). Although we described the courtship behaviour of *S. perspicillata* as observed just in one population, however our data agree with the published (but less detailed or fragmentary) and unpublished data (S.J. Arnold) from other populations. and thus they are relatively robust and generalisable.

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