Web-building behaviour in *Araneus diadematus* Cl.: Effects of temperature and neurotoxins on the behaviour and web-geometry

M.Sc. Thesis (Speciale)
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Preface

This thesis has been submitted to the Institute of Biology, University of Aarhus, Denmark in fulfilment of the requirements to obtain the “Cand Scient. Biol.” (M.Sc.) degree. The work presented in this thesis has been carried out at the Department of Zoology under the supervision of Fritz Vollrath (external supervisor, University of Oxford) and Mark Bayley (internal supervisor).

Besides the invaluable help I have received from my supervisors, I have also been fortunate to discuss my ideas with Samuel Zschokke during a brief visit to the Department for Integrative Biology, University of Basel, Switzerland at the beginning of my master studies. I would also like to thank Else Bomholt and Per Henriksen for their technical help and advice with the experiments and David Mayntz for his advice concerning the use of statistic. Credits are also due to Cedric Dicko and Lennart Kiil for their comments on part of this thesis.

Lastly, I’m grateful for the comments and stimulating discussions I’ve had with the persons I shared office with for shorter or longer periods. Cedric Dicko, Lisette Jespersen, Ann Kyneb, Johannes Lomborg, David Mayntz, Thomas Nørgaard, Henrik Rasmussen and Jeanette Vollmer.

Thomas Hesselberg
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Chapter 1: Introduction

Outline and Aims

This thesis consists of 5 chapters. The first is an introductory chapter in which aims and outline are presented, as well as some background information regarding the model organism, the methods and drugs used. The chapters 2-4 then present the results obtained during my master study in article format. In chapter 2 data regarding the effects of temperature on the predator-prey system is presented. Chapter 3 consists of a short article concerning the effects of temperature on web-geometry and web-building. The main part of this study comprising the effects of drugs on web-geometry and web-building behaviour can be found in chapter 4. Finally a short discussion is given in chapter 5 where the findings and shortcomings of the three previous chapters are put into perspective. References can be found at the end of each chapter. The article-format was chosen for presenting the findings, since this is the standard way of presenting new results in science and, furthermore, because it allows readers to selectively read these articles independent of the rest of the thesis.

When studying ethology it is worth considering the choice of experimental animal carefully (Martin and Bateson 1993). Here web-building spiders present a unique model organism, since the web can be considered a solidified record of a complex movement pattern and reveal information about the operations of the sensory and motor apparatus (Vollrath 1992; Witt and Reed 1965). Due to the orb web's two-dimensional structure and its highly regular geometry, it can easily be photographed and analysed. In this study I have used the orb-weaver *Araneus diadematus* as a model organism to design experiments with the aim of increasing our knowledge of the laws governing web-building behaviour in orb-weavers. Successful computer-simulations of orb-web construction have shown that the spider follows relatively simple rules of behaviour, when building a web (Krink & Vollrath 1997; Gotts & Vollrath 1991). However, these rules are still poorly known and only through the interplay between further more advanced computer simulations and experiments with real spiders can we hope to gain a fuller understanding of the mechanisms behind orb-web construction. The study presented here attempts to answer the question of how changes in external (temperature) and internal (change of function of the central nervous system due to neurotoxins) factors influence the behaviour pattern (i.e. building a web). This approach will, without analysing the exact neurophysiological processes, generate information about the importance of these factors on the various aspects of web-building behaviour. Furthermore, aspects of the predatory behaviour were dealt with in experiments on prey escape time and the spiders' capture speed at different temperatures.

Although the experiments in this study were all laboratory based, they were still considered relevant in a more ecological and adaptational sense. The chosen temperatures were of ecological relevance, since *Araneus diadematus* is exposed to them during its life in nature. By viewing the spider and its web as an integrated prey-catching system, it is possible to see how this system is adapted to different temperatures. At first glance the drug experiments may not seem very connected to the life of spiders in nature, although changes in web-geometry due to pesticides have been found (Samu et al. 1992; Samu & Vollrath 1992). However, the choice of neurotoxins ensured that some senses and brain functions were knocked out during the experiment. Even though it was beyond the scope of this study to verify if and which senses actually were targeted by the specific drug, it was possible to make assumptions based on observations of changes in behaviour.

The Natural History of the Cross Spider

The cross spider *Araneus diadematus* (Clerck 1757) is one of the best known and most common species in the orb-weaving family Aranidae. It is easily found and identified in the field due to its large conspicuous webs and the characteristic white cross on the abdomen. The spider occupies a wide range of habitats, but is most frequently found on bushes and other vegetation on heath land (Roberts 1995). The cross spider can, however, also be found in gardens, giving rise to another common name for this species, the (European) garden spider. This name is, although, somewhat misleading, as the spider is much more frequent on heath land and is not among the most common spiders found in gardens (Roberts 1995). The first webs appear in early May and are made by overwintering juveniles. Two overwintering strategies are used by the cross spider, the summer generation overwinter as juveniles and the fall generation overwinter in the egg-sac. The cross spider, like most *Araneus* species, is very resistant to cold (Kirchner 1973). The emerging spiderlings reach maturity during late summer and early fall after 3-4 moults for males and 5-6 for females (Ramousse 1973).

The adult females are much larger than the adult males and whereas the females can live up to eight months in the lab the males die shortly after their last moult (Witt 1971). Adult males do not build webs and instead spend their short adult life in active search of females (Ramousse 1973). Although the male and female show large differences in body size and growth in late life, the males can only be separated from the females between the last two moults (Ramousse 1973) and studies show that the early growth of both sexes are similar (Witt et al. 1972). The cross spider builds its web in the early morning, when the temperature is at its minimum (Spronk 1935) and spends the day motionless waiting for prey. Juvenile spiders are often sitting in the hub during the day, whereas adult females can also be found hidden in retreats located in the surrounding vegetation while connected to the hub by a signal thread (pers. obs.). As the majority of spiders, *Araneus diadematus* is a general predator and will attack most of the insects hitting the web (Reichert and Luczak 1982).

The orb-web

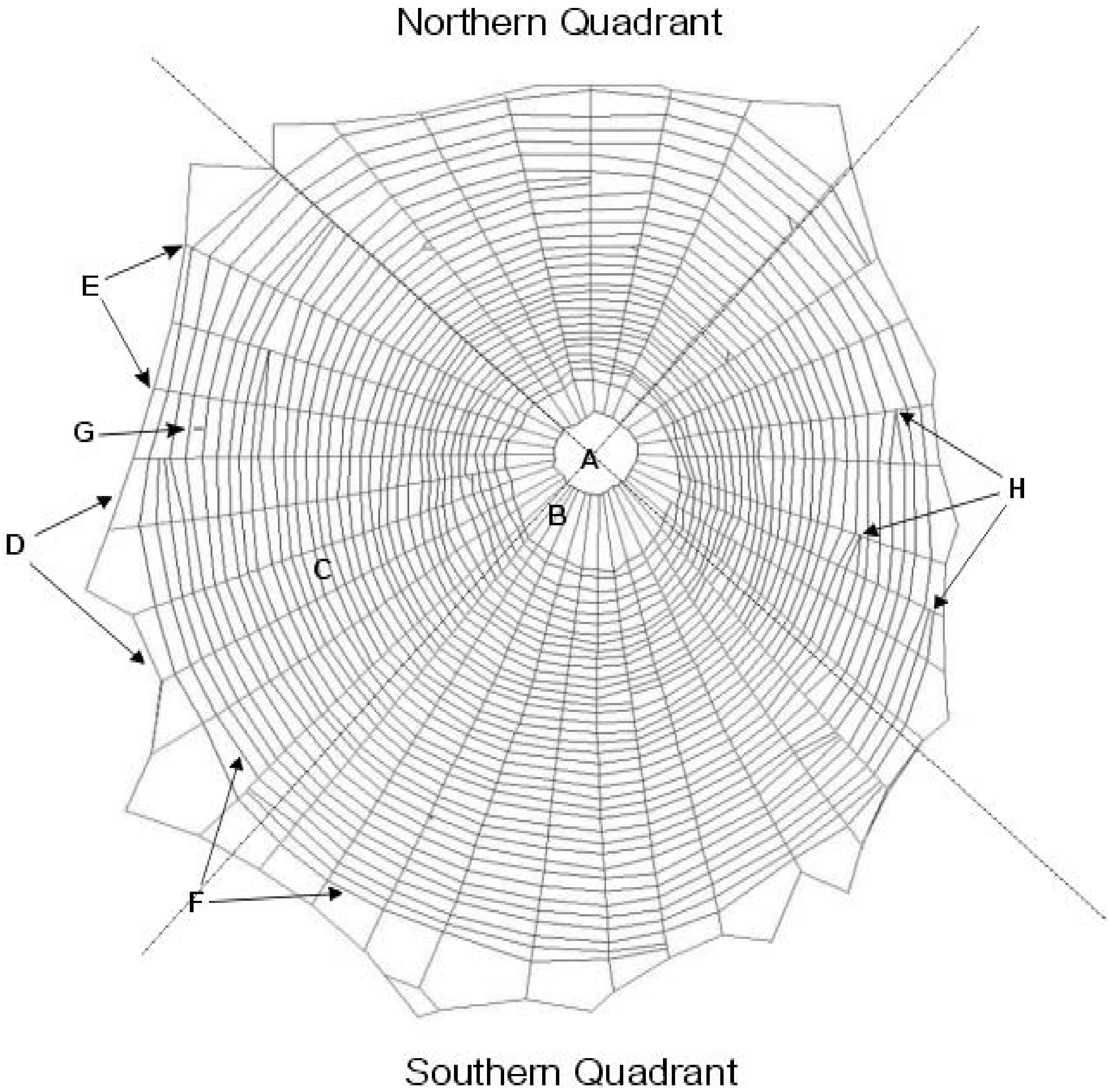


Fig. 1. A digitised web of *Araneus diadematus* with letters referring to parts of special interest. The crossing lines split the web into 4 quadrants with the northern and southern quadrant emphasised. A) the hub, B) the free sector, C) the capture spiral, D) the frame, E) radii, F) spiral turns, G) mesh size and H) reverses. See the text for further explanations of these terms.

When people talk about spider webs, they most often refer to the orb-web. This characteristic web type is the best known and also the most conspicuous in nature. Orb webs are built by more than 2000 species found among both ecribillate and cribillate spiders (Foelix 1996). Here, as in the rest of this thesis, attention is focused on *Araneus diadematus*, but although species specific differences are found in the webs (Eberhard 1990; Risch 1977), these are often only minor differences in the detailed geometry and symmetry of the webs. Therefore, the parts and structures described in this section are generally found in all orb-webs, except for special cases, such as the ladder spider *Scoloderus* and bolas spider *Mastophora* (both in the family Araneidae), where the web has been extremely reduced and modified to allow specialisation for certain prey types (Heiling and Herberstein 2000; Foelix 1996).

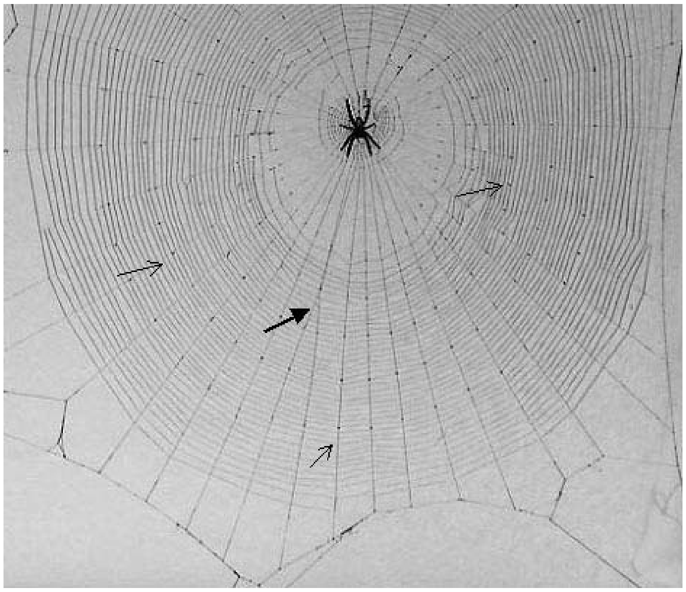


Fig. 2. Inverted photo of the southern half of a web of *Araneus diadematus*. The spider is sitting in the hub in the waiting-position. The thin arrow points to the remains of the auxiliary spiral. The thick arrow points to a Y-shaped radius.

Since the early studies on web-geometry a great number of different names have been used for the same structures, thus sometimes making comparisons between different studies tedious. In the following, I use the nomenclature recommended by Zschokke

(1999), although, I apply capture spiral instead of sticky spiral and reverses instead of U-turns. The web is traditionally divided into 4 quadrants each covering a 90° angle (see Fig. 1). The orb-web is asymmetrical with the southern quadrant being larger, having a more even mesh size and more radii than the other quadrants (ap Rhisiart and Vollrath 1994; Vollrath and Mohren 1985). In the centre of the orb web is the hub (see Fig. 1A), which in *Araneus diadematus* consists of a spiral interwoven with a criss-cross of threads. The spider often sits in the hub in a typical waiting position with the cephalothorax pointing downward ready to attack intercepted prey (see Fig. 2). Outside of the hub is the free sector (see Fig. 1B), which is a zone with no threads except the converging radii. Following that, we have the capture spiral (see Fig. 1C), which, as the name implies, consists of sticky silk and is used to catch and detain incoming prey. The frame is the outer limit of the web (see Fig. 1D) and is attached to the surroundings with anchor threads (not shown in Fig. 1). Another important component of the web is the auxiliary spiral, but this spiral is in *A. diadematus* and most other orb-weavers only a temporary spiral, which is removed during capture spiral-building (see next section). However, small bundles of threads are left as remains and these are usually visible in the final web (see Fig. 2). The area within the frame is called the web area, whereas the capture area is the area covered by the capture spiral (excluding the area of the free zone and the hub). A normal web of *A. diadematus* consists of around 30 radii (Vollrath et al. 1997; Witt and Read 1965) that run from the hub to the frame (see Fig. 1E). If a radius splits into two before reaching the frame, it is referred to as a Y-shaped radius. In this thesis only radii splitting into two inside the capture area were recognised as Y-shaped radii (see Fig. 2). Each loop of the capture spiral is called a spiral turn (see Fig. 1F) and the mesh size is the distance between two consequent spiral turns (see Fig. 1G). During capture spiral-building the spider changes the direction of thread laying several times (see next section) and the resulting characteristical V-endings are known as reverses (see Fig. 1H).

Web-building

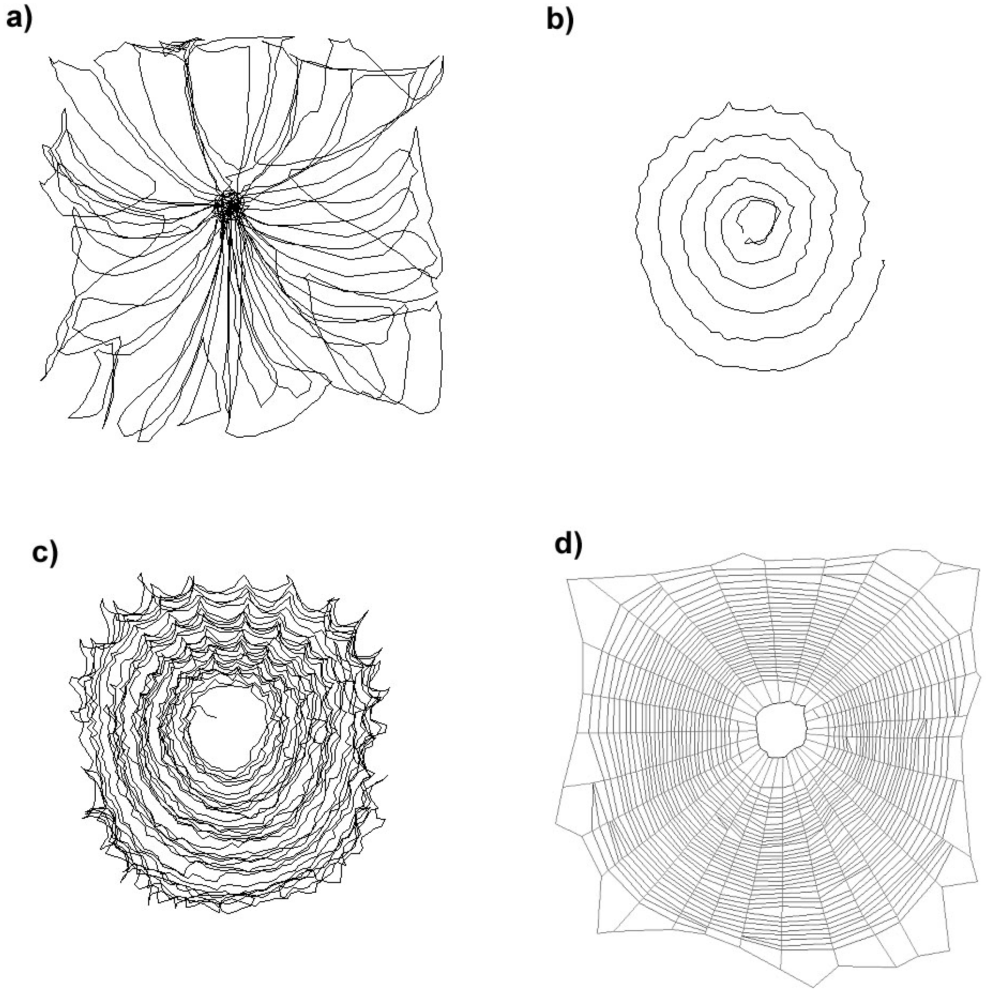


Fig 3. The movement pattern exhibited by *Araneus diadematus* during the three major stages of orb-web construction. a) Construction of the frames, hub and radii. b) Construction of the auxiliary spiral. c) Construction of the capture spiral. d) The finished web.

It is puzzling how spiders, considering their limited mental capacity, can construct such a complicated structure as the orb-web. However, accurate simulations have shown that web-building may require no more than a few basic rules (Krink and Vollrath 1999). During web-building the spiders seemingly follow an innate programme, which shows only little plasticity. Manipulations of spiders and webs during web-construction reveal that the neural programme is not followed strictly from beginning to end, but depends on continuous feedback from the emerging web (Peters 1970). Traditionally, experience and learning have been viewed as playing no role in web-building. Reed and co-workers (1970) showed that preventing spiders from building webs early in life made no difference to their adult webs as compared to normal adult webs. However, a recent study by Heiling and Herberstein (1999) has challenged this view. They found that inexperienced spiders build more symmetric webs, with less investment in the southern part, compared to experienced spiders. This is in agreement with the observation that the first webs from juvenile spiders generally resemble webs build by older spiders, but are more circular (Mayer 1953).

The construction of the web always follows a strict order (Foelix 1996; Vollrath 1992, Peters 1939). This web-building sequence can, for *Araneus diadematus*, be divided into 6 more or less distinct stages (Gotts and Vollrath 1991). *Stage 0:* Exploration of the surroundings. The spider moves around a potential website while trailing a silken drag-line. This is a very important stage where the future position and planarity of the web are determined (Vollrath 1992). *Stage 1*: Creation of the hub and the first radii. The spider starts by placing an initial bridge thread between two points, then moves to the centre of the bridge thread and attaches a new thread from there to a point further down. This creates a Y-structure, which essentially is the basic framework, with the future location of the hub in the centre of the three radii (Peters 1939). This stage is often skipped when the spider builds further webs at the same site. *Stage 2*: Completion of the frame, hub and radii. Construction of the following radii and the frame occurs simultaneous with an enlargement of the hub until the entire frame is build. Lastly, extra radii are added to fill out the gaps. The placement of these is determined by the spider by turning around in the hub and measuring the gap between neighbouring radii with its front legs and whenever the gap is too large a new radius is inserted. The spider does this by moving out an existing radius and moving along the frame until it attaches the dragline, whereby a temporal radius is built. The spider then moves along this temporal radius to the hub while replacing the temporal radius with a permanent radius. This creates the leaf-shaped movement-pattern recorded by computer-automated video tracking (see Fig.3a). Subsequent radii are generally placed opposite each other, although more radii usually are found in the southern half (ap Rhisiart and Vollrath 1994). *Stage 3*: Construction of the auxiliary spiral. When no new gaps are found, the spider proceeds to build the auxiliary spiral. It resembles a logarithmic spiral (Vollrath and Mohren 1985), with progressively larger distance between each spiral turn (see Fig. 3b). The auxiliary spiral is built from the hub and outwards in one smooth movement, with reverses being extremely rare (Zschokke and Vollrath 1995a). It is constructed using the radii as a scaffold and, at least for another orb-weaver *Leucage mariana*, previously laid auxiliary spiral turns serve as guide for the placement of current threads (Eberhard 1988). The auxiliary spiral stabilises the radii structure, and prevents large sagging of the radii when the spider moves across them (Peters 1970). The auxiliary spiral is removed during building of the capture spiral. *Stage 4*: Construction of the capture spiral. After a brief pause at the hub the spider commences to build the capture spiral (see fig. 3c). The capture spiral is an arithmetic spiral with nearly equal distances between the subsequent turns of the spiral (Vollrath and Mohren 1985). The spiral is constructed from the periphery towards the hub and consists entirely of sticky silk. The capture spiral, contrary to the auxiliary spiral, is not continuous, but consists of numerous reverses and gaps. The auxiliary spiral and the previously laid capture spiral turns are used as guides during construction (Zschokke and Vollrath 1995a; Zschokke 1993; Vollrath 1986). This is the most time-consuming stage and takes more than 2/3 of the around 30 minutes needed for *Araneus diadematus* to build an orb-web. *Stage 5*: Final adjustments. The spider stops building the capture spiral some distance away from the hub, but continues to remove the auxiliary spiral thereby creating a free zone. It finishes with biting out the centre of the hub to replace it with a number of criss-crossing threads.

Araneus diadematus readily builds its web in total darkness (Spronk 1935), so visual cues play no role during web-construction. Instead the spiders rely on other cues. Experiments with vertical rotation in a klinostat show that gravity plays an important role in orientation during web-building (Vollrath 1988; Vollrath 1986). However, since spiders are capable of building webs, albeit irregular ones, in the absence of gravity (Witt et al. 1977), other mechanisms must be available. One of these could be the use of idiothetic memory (Vollrath et al. 2002; Vollrath 1992). However, our knowledge of the orientation mechanism used during web-building is very limited.

Predatory behaviour

Although the web has a variety of different functions such as detecting mates and enemies, its main function is to intercept and detain prey long enough for the spider to catch it. The structure of the orb-web is optimised for prey capture (Krink and Vollrath 2000; ap Rhisiart and Vollrath 1994; Eberhard 1986). A comparative study with tangle webs and sheet webs, showed that orb webs intercept more prey and detain a greater proportion of the intercepted prey (Rypstra 1982). However, the web cannot be viewed as a simple trap that the spider can empty at its leisure. Most types of prey will escape from the web sooner or later (Nentwig 1982), therefore a successful capture depends on the behaviour of the spider.

The predatory behaviour of the spider can be described as a rigid sequence of fixed behavioural patterns (Reichert and Luczak 1982), although slight differences are found between species (Weissmann and Vollrath 1999; ap Rhisiart and Vollrath 1994; Robinson et al. 1969) and for different prey-types (Suter 1978; Robinson et al. 1969). The spider will usually be sitting in the hub in a characteristic waiting position with the cephalothorax turned downward (see Fig. 2) or in a retreat connected to the hub with a signal thread. Here it sits motionless while continuously monitoring the web, waiting for prey to be intercepted by the web. When this happens, the spider's resulting behaviour can be divided into 5 discrete steps (Weissmann and Vollrath 1999). *Step 1*: Receiving the vibratory stimulus generated by the prey and reacting to it by turning towards it and move 10 or 20 mm in its direction (ap Rhisiart and Vollrath 1994). In this study, the time needed to complete this step is called reaction time. *Step 2:* Confirming direction of prey and getting information about distance to and size of prey, by plucking the radii violently with the first pair of legs (Weissmann and Vollrath 1999). The time spent in this step is here called orientation time. *Step 3*: Running towards the prey. In some cases the spider pauses en route or passes the prey before finally catching it (pers. obs.). The time from leaving the hub until touching the prey with its chelicerae is here referred to as capture time. *Step 4:* Attacking the prey. Depending on size and weaponry of prey, it is either wrapped first and then bitten or just simply bitten (Robinson et al. 1969). *Step 5:* Transportation of prey back to the hub. Large prey is wrapped before being moved to the hub, making it easier to transport (Robinson et al.1969), whereas smaller prey such as *Drosophila* usually is carried in the chelicerae (pers. obs.). In cases where several prey are intercepted by the web simultaneously or in rapid succession, the spider often leaves wrapped prey at the capture site and returns directly to the hub (pers. obs.).

General methods

The exact methods used for each experiment are given in the relevant chapters. This section is only meant to provide background details and a limited discussion of the methods employed throughout the study presented in this thesis. Juvenile *Araneus diadematus* were collected on junipers (*Juniperus communis*) near the Mols Laboratory in Eastern Jutland in spring and summer 2001 and spring 2002. Some further individuals were collected in fall 2001 and in summer 2002 on a hedge surrounding a cemetery in central Århus. Too small spiders were reared in the laboratory under standard conditions (25° ± 2°C; 45% ± 5% rH, 16/8 L/D) on a saturation diet of normal and high-protein fruit flies (*Drosophila melanogaster*). Spiders used for experiments weighed approximately 20 to 60 mg with a body length from 5 to 10 mm. Only juvenile spiders in instar 3,4 or 5 were used, since they do not allocate energy to reproduction and therefore are expected to build more regularly and with less day-to-day variation in web-geometry. When not used for experiments spiders were kept in small vials in the fridge at 10 °C. Once a month they were taken out and fed 3-4 fruit flies and given water. Before used in experiments spiders were taken out and allowed at least one week of acclimatisation under standard lab conditions (25° ± 2°C; 45% ± 5% rH, 16/8 L/D). Orb-weavers can show an individual difference in web-geometry between subsequent webs (Sherman 1994) and therefore special attention is needed to keep housing and diet well controlled (Vollrath and Samu 1997). In the laboratory, spiders were individually placed in 30x30x5 cm Perspex frames and stacked like books in a bookshelf separated with thin Vaseline smeared plastic sheets. Frames were watered daily and had the spider built a web, one fruit fly was given and the web was cut using a Turbo Solderer. This was done to enhance the probability that the spider built a new web the following day. Webs were cut leaving an intact radius in the north and in the south part resulting in the collapse of the web. *Araneus diadematus* recycles around 90% of silk proteins from the old web, when constructing a new one (Peakall 1971). The silk was, therefore, left in the frame for the spider to ingest, thereby encouraging the spider to rebuild its web. As spiders were only fed if they had built a web, there was variation between the spiders in their nutritional state, but since spiders were used as their own controls this should have no effect on the results obtained. The reason for only feeding spiders that had built a web, was first of all that mouth-feeding all the spiders would have been too time-consuming, but also as a precaution to avoid overfeeding the spiders, since satiated spiders are less inclined to build webs (Vollrath and Samu 1997). However, since starved spiders show increased variation in web-geometry of successive webs (Vollrath and Samu 1997), only healthy looking spiders were used in experiments.

In earlier days elaborate techniques were developed for obtaining photographs of the webs (Witt 1971). Today progress in photographic equipment has made the process somewhat simpler. The webs were placed in the opening of a black box equipped with lights illuminating the web from the sides. To gain the best contrast no further sources of light was in the room. A Nikon Coolpix 995 digital camera was used to photograph the webs. The camera was set to record black and white photographs with an image quality of fine (JPEG with a compression ratio of 1/4). The image size was set to XGA (1024 x 768 pixels). Best photographs were obtained when the shutter speed was set to 2 sec. and aperture to 3.5. The images were then directly downloaded to the computer and analysed using the software Web Extractor for the Macintosh (Pedersen 1995).

Recording of the behaviour exhibited during web-building was done by employing computer-automated video tracking. This technique has been used successively for analysing spider locomotion (Baatrup and Bayley 1993) and web-building (Benjamin and Zschokke 2002; Zschokke and Vollrath 1995a, b). The frame containing the spider was placed in front of a high contrast background and recorded by a black and white Panasonic surveillance camera. The video image was then scanned by the scanning unit 'VP112' (Baker box) to detect the object. The position of the object was transferred to a computer running the software Move Recorder for the Macintosh (Zschokke 1994), which recorded the positions as x-y co-ordinates and stored them on the computer. New positions were only recorded when the spider had moved a certain threshold distance. The recorded movement pattern was analysed using Move Watch for the Macintosh (Zschokke 1994).

Several options are available for administering drugs to spiders: Injection, topical and oral administration as well as inhalation (Witt and Reed 1965). Since inhalation is only convenient for volatile compounds and injection is very difficult in small hard-bodied animals without causing high mortality (Wolff and Hempel 1951), these methods were quickly rejected. Comparisons between topical and oral administration revealed no differences in effect for most drugs (Witt and Reed 1965), although see Samu and Vollrath (1992). Since oral application were favoured in earlier studies (Witt 1971; Witt and Reed 1965) and seemed more convenient for web-building spiders, it was chosen for this study. Spiders were removed from their webs, without destroying the webs, and weighed with an accuracy of 0.01 mg. They were then allowed some time to settle down in the hub before drugs were applied. A weight-dependent dose was given as one or several drops to the mouthparts of the spider using a Hamilton syringe. The drugs were dissolved in water and sugar was added to ensure that spiders actively accepted and ingested the drops. After 30 to 45 minutes when all traces of liquid were gone from the spider's mouthparts, the spiders were re-weighed and the ingested amount calculated. In no cases did the weight gain match the amount of solution administered, so some was either wiped off by the spider or regurgitated before weighing. This meant that it was impossible to control the exact amount of drug imbibed by the spider. However, this was not important for the experiments described in this thesis, since emphasis was on the qualitative effects of the neurotoxins.

A brief introduction to the neurotoxins used

Scopolamine

Description: Scopolamine hydrobromide trihydrate ($C_{17}H_{22}BrNO_4{\bullet}3H_2O$), also known, as l-hyoscine is a naturally occurring tropane alkaloid found in a number of species in the family Solenaceae (Merck Index 1989). Due to its great solubility scopolamine is one of the major active alkaloids and can, in some species, occur in relatively high concentrations. Shonle and Bergelson (2000) report that *Datura stramonium* contains on average 1.55 mg scopolamine per g dry leaf.

Mechanisms of action: Scopolamine has anticholinergic effects, i.e. it blocks the receptors of the neurotransmitter acetylcholine. The brain contains two types of cholinergic receptors; the muscarinic and the nicotinic receptor. The muscarinic receptors are predominantly found in the CNS, while nicotinic receptors are more prevalent in skeletal muscles and the main ganglia (Bradford 1986). Scopolamine antagonises acetylcholine at the muscarinic receptors, but in high doses it has been found to antagonise nicotinic receptors as well (Kopelman 1986).

Effects: Scopolamine is used as medicine to prevent motion sickness. At therapeutic doses it produces CNS depression resulting in drowsiness and amnesia, however at higher doses it can lead to CNS excitation resulting in hallucinations or delirium. Due to its anticholinergic action, scopolamine is used to induce memory impairment in humans (Riedel et al. 1995). It has a well-documented negative effect on memory and learning in humans and monkeys (Harder et al. 1998; Kopelman 1986). A Study on octopuses reveals a similar effect, with scopolamine injections resulting in an impairment of visual learning (Fiorito et al. 1998). However, a study on the cockroach shows no effect of scopolamine on learning or retention, although an effect on habituation is found (Barraco and Eisenstein 1984). None of the studies report any adverse effects on behaviour. Scopolamine is found naturally as a secondary compound in some plant species and leads to higher mortality in the insect larvae *Spodoptera littoral* (Krug and Prokosch 1993), although it apparently acts as a phagostimulant for *Epitrix* flea beetles (Shonle and Bergelson 2000).

In the 50s and 60s several drugs were administered to orb-weaving spiders in order to see how these affected the web-geometry (Witt and Reed 1965). Scopolamine is among the drugs tested on adult *Zygiella x-notata* (Witt and Reed 1965; Wolff and Hempel 1951). The studies report the following effects: 1) No change in overall web-building frequency. 2) A decrease in web area and capture spiral area. 3) An increase in length/width ratio, resulting in higher eccentricity. 4) High doses lead to systematic disruptions in the capture spiral and hub, occasionally even to the occurrence of more centres. It is hypothesised that scopolamine disrupts the orientation ability in spiders, without affecting motor and sensory skills (Wolff and Hempel 1951).

Amphetamine

Description: Amphetamine is the prototype for a family of synthetically produced drugs. The two most widely used amphetamines are dextroamphetamine sulphate (d-amphetamine: $C_{18}H_{28}N_2O_4S$), also known as dexedrine, and methamphetamine hydrochloride ($C_{10}H_{16}ClN$), also known as pervitin, which have similar modes of action (Davidson et al. 2001; Merck Index 1989).

Mechanisms of action: Amphetamines are necrotic neurotoxins, which kill neurons directly through the production of free radicals (Frost and Cadet 2000). However, recently it was found that they also result in apoptic cell death, killing neurons by triggering a mitochondria dependent induction of internal cell suicide (Davidson et al. 2001). Methamphetamine primarily destroys populations of dopaminergic and serotonergic (5HT-ergic) terminals causing release of the neurotransmitters dopamine and serotonine (Davidson et al. 2001; Frost and Cadet 2000).

Effects: In humans the amphetamines act on the sympathetic and central nervous system with a potent psychostimulant action, resulting in euphoria, increased alertness, initiative and confidence and a lessened sense of fatigue. Especially methamphetamine is widely used as an illegal stimulant and is sold on the black-market under the name "ice" or "speed" (Frost and Cadet 2000). Due to their effect of increasing concentration amphetamines are used in connection with hyperactive children (Merck Index 1989). Amphetamines also show effects on animals. D-amphetamine induces an amnesia-like effect in rats, however, this might be due to disturbances in the sensory motor system (Kaminsky et al. 2001). Kusayama and Watanabe (2000) have shown that planarians increased their staying time in otherwise non-preferred patches when these were associated with methamphetamine administration. They suggest that this indicates a similarity in the stimulating effects of methamphetamine in humans and planarians. No reliable information is available on how amphetamines affect spider behaviour, however, it has been suggested that methamphetamine disrupts the sensory control of *Zygiella x-notata* (Peters et al 1950). Spiders have been observed to reach into empty air during capture spiral construction when keeping contact with the previous turn of the spiral or to choose an abnormally small or large distance to it. Amphetamines cause changes in orb-web geometry in both *Zygiella x-notata* and *Araneus diadematus* (Witt et al. 1968; Witt and Reed 1965; Peters et al. 1950). The reported effects seem to be dose-dependent with low dosage resulting in an increase in web-building frequency and an increase in web size. High dosage causes a decrease in web-building frequency, a decrease in web-size and a decrease in capture spiral regularity.

Caffeine

Description: Caffeine, with the chemical name trimethylxanthine ($C_8H_{10}N_4O_2$), is an alkaloid and occurs primarily in coffee, tea, cacao beans and cola nuts (Merck Index 1989). It is structurally and chemically related to theophylline, the active ingredient in tea.

Mechanisms of action: Caffeine antagonises adenosine receptors at low concentrations, but at higher concentrations it also acts as an inhibitor of cyclic nucleotide phosphodiesterase activity (Sawynok and Yaksh 1993).

Effects: Caffeine has a stimulant effect on motor and mental activities in humans (Sawynok and Yaksh 1993). It improves motor performance, decreases fatigue and increases mental alertness. Caffeine also acts to reduce pain, especially in combination with other pharmaceuticals (Sawynok and Yaksh 1993). It has, furthermore, been shown to possess memory enhancing effects (Riedel et al. 1995). However, caffeine is mildly addictive and withdrawal effects include drowsiness, decreases in mood, and headaches (Rogers et al. 1995). Caffeine stimulates psychomotor activity and performance at low doses in rats and mice, but at higher doses a reduced effect is observed (Sawynok and Yaksh 1993). In *Drosophila*, caffeine had no effect on general behaviour (Dudai et al. 1986), but resulted in significantly reduced visual learning performance (Folkers and Spatz 1984). Caffeine is one of the most powerful drugs to induce changes in web-geometry among the numerous tested on spiders (Witt et al. 1968; Witt and Reed 1965; Peters et al. 1950). In webs of mature *Zygiella x-notata*, it causes the following changes without affecting web-building frequency. 1) A decrease in capture spiral area. 2) A change in eccentricity to more round webs. 3) An increase in the number of radii not running the full length from hub to frame. Furthermore, total construction time has been observed to nearly double under the influence of caffeine.

References

Ap Rhisiart, A.; Vollrath, F. 1994. Design features of the orb web of the spider, *Araneus diadematus*. *Behavioral Ecology* **5:**280-287.

Barraco, D. A.; Eisenstein, E. M. 1984. Effects of Pre-training administration of scopolamine on learning and retention in the cockroach, *P. americana*. *Pharmacology, Biochemistry & Behavior* 20: 479-481.

Baatrup, E.; Bayley, M. 1993. Quantitative analysis of spider locomotion employing computer-automated video tracking. *Physiology and Behavior* **54**: 83-90.

Benjamin, S.; Zschokke, S. A computerised method to observe spider web-building behaviour in a semi-natural light environment. From *European Arachnology 2000* (S. Toft and N. Scharff eds.) pp 117-122. Aarhus University Press, Aarhus, 2002.

Bradford, H. F. *Chemical neurobiology: An introduction to neurochemistry.* W. H. Freeman and Company. New York 1986.

Davidson, C.; Gow, A. J.; Lee, T. H.; Ellinwood, E. H. 2001. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. *Brain Research Reviews* **36:** 1-22.

Dudai, Y.; Buxbaum, J.; Corfas, G.; Orgad, S.; Segal, D.; Sher, B.; Uzzan, A.; Zvi, S. 1986. Defective cAMP metabolism and defective memory in Drosophila. *Acta Biochem. Biophys. Hung.* **21**: 177-192.

Eberhard, W. G. 1986. Effects of orb-web geometry on prey interception and retention. From William A. Shear (ed.) *Spiders: Webs, behaviour and evolution.* p. 70-100.

Eberhard, W. G. 1988. Memory of distances and directions moved as cues during temporary spiral construction in the spider *Leucage mariana* (Araneae: Araneidae). *Journal of Insect Behavior* **1**: 51-66.

Eberhard, W. G. 1990. Function and phylogeny of spider webs. *Annual Review of Ecology and Systematics* **21**: 341-372.

Fiorito, G.; Agnisola, C.; d'Addio, M.; Valanzano, A.; Calamandrei, G. 1998. Scopolamine impairs memory recall in *Octopus vulgaris*. *Neuroscience Letters* **253:** 87-90.

Foelix, R. F. *Biology of spiders 2nd Ed*. Oxford University Press, Oxford. 1996

Folkers, E.; Spatz, H. C. 1984. Visual learning performance of *Drosophila melanogaster* is altered by neuropharmaca affecting phosphodiesterase and acetylcholine transmission. *Journal of Insect Physiology* **30**: 957-965.

Frost, D. O.; Cadet, J-L. 2000. Effects of methamphetamine-induced neurotoxicity on the development of neural circuitry: a hypothesis. *Brain Research Reviews* **34:** 103-118.

Gotts, N. M.; Vollrath, F. 1991. Artificial intelligence modelling of web-building in the garden cross spider. *Journal of Theoretical Biology* **152**: 485-511

Harder, J. A.; Baker, H. F.; Ridley, R. M. 1998. The role of the central cholinergic projections in cognition: implications of the effects of scopolamine on discrimination learning by monkeys. *Brain Research Bulletin* **45:** 319-326.

Heiling, A. M.; Herberstein, M. E. 1999. The role of experience in web-building spiders (Araneidae). *Animal Cognition* **2**: 171-177.

Heiling, A. M.; Herberstein, M. E. 2000. Interpretations of orb-web variability: A review of past and current ideas. *Ekología* **19:** 97-106.

Kaminsky, O.; Klenerova, V.; Stöhr, J.; Sida, P.; Hynie, S. 2001. Differences in the behaviour of Sprague-Dawley and Lewis rats during repeated passive aviodance procedure: effects of amphetamine. *Pharmacological Research* **44**: 117-122.

Kirchner, W. 1973. Ecological aspects of cold resistance in spiders (a comparative study). From *Effects of temperatures on ectothermic organisms*. Ed. Wieser, W. Springer-Verlag Berlin.

Kopelman, M. D. 1986. The cholinergic neurotransmitter system in human memory and dementia: A review. *The Quarterly Journal of Experimental Psychology* **38A**: 535-573.

Krink, T.; Vollrath, F. 1997. Analysing spider web-building behaviour with rule-based simulations and genetic algorithms. *Journal of Theoretical Biology* **185**: 321-331

Krink, T.; Vollrath, F. 1999. A virtual robot to model the use of regenerated legs in a web-building spider. *Animal Behaviour* **57**: 223-232.

Krink, T.; Vollrath, F. 2000. Optimal area use in orb webs of the spider *Araneus diadematus*. *Naturwissenschaften* **87**: 90-93.

Krug, E.; Proksch, P. 1993. Influence of dietary alkaloids on survival and growth of *Spodoptera littoralis*. *Biochemical Systematics and Ecology* **21:** 749-756.

Kusayama, T.; Watanabe, S. 2000. Reinforcing effects of methamphetamine in planarians. *NeuroReport* **11**: 2511-2513.

Martin, P.; Bateson, P. *Measuring Behaviour: An introductory guide 2nd Ed.* Cambridge University Press. 1993.

Mayer, G. 1953. Untersuchungen über herstellung und struktur des radnetzes von *Aranea diadema* und *Zilla x-notata* mit besonderer berücksichtigung des unterscheides von jugend- und altersnetzen. *Zeitschrift für Tierpsychologie* **9**: 337-362.

Merck Index. An encyclopedia of chemicals, drugs and biologicals. Edited by Susan Budavari. Merck & Co., Inc. Rahway, New Jersey, USA 1989.

Nentwig, W. 1982. Why do only certain insects escape from a spider's web? *Oecologia* **53**: 412-417.

Peakall, D. B. 1971. Conservation of web proteins in the spider, *Araneus diadematus*. *Journal of Experimental Zoology* **176**: 257-264.

Pedersen, P. O. 1995. Web Extractor: Processing of spider web images. *M.Sc. Thesis*. Department of Computer Science, University of Aarhus, Denmark.

Peters, H. M. 1939. Über das kreuzspinnennetz und seine probleme. *Naturwissenschaften* **47:** 777-786

Peters, H. M.; Witt, P. N.; Wolff, D. 1950. Die beeinflussung des Netzbaues der Spinnen durch Neurotrope Substanzen. *Zeitschrift für Vergleichende Physiologie* **32**: 29-45.

Peters, P. J. 1970. Orb-web construction: interaction of spider (*Araneus diadematus* Cl.) and thread configuration. *Animal Behaviour* **18**: 478-484.

Ramousse, R. 1973. Body, web-building and feeding characteristics of males of the spider *Araneus diadematus* (Araneae: Araneidae). *Psyche* **80**: 22-47.

Reed, C. F.; Witt, P. N.; Peakall, D. B. 1970. Experience and the orb-web. *Developmental Psychobiology* **3**: 251-265.

Reichert, S. E.; Luczak, J. Spider foraging: Behavioral responses to prey. From *Spider communication mechanisms and ecological significance*. Eds. Witt, P.N and Rovner, J. S. Princeton University Press. 1982.

Riedel, W.; Hogervorst, E.; Leboux, R.; Verhey, F.; van Praag, H.; Jolles, J. 1995. Caffeine attenuates scopolamine-induced memory impairments in humans. *Psychopharmacology* **122**: 158-168.

Risch, P. 1977. Quantitative analysis of orb web patterns in four species of spiders. *Behavior Genetics* **7**: 199-238.

Roberts, M. J. *Spiders of Britain and Northern Europe.* Collins field guide. HarperCollins*Publishers* Bath 1995.

Robinson, M. H.; Mirick, H.; Turner, O. 1969. The predatory behavior of some araneid spiders and the origin of immobilization wrapping. *Psyche* **70**: 487-501.

Rogers, P. J.; Richardson, N. J.; Dernoncourt, C. 1995. Caffeine use: is there a net benefit for mood and psychomotor performance? *Neuropsychobiology* **31**: 195-199.

Rypstra, A. L. 1982. Building a better insect trap; an experimental investigation of prey capture in a variety of spider webs. *Oecologia* **52**: 31-36.

Samu, F.; Matthews, G. A.; Lake, D.; Vollrath, F. 1992. Spider webs are efficient collectors of agrochemical spray. *Pesticid Science* **36**: 47-51.

Samu, F.; Vollrath, F. 1992. Spider orb web as bioassay for pesticide side effects. *Entomol. Exp. Appl.* **62**: 117-124.

Sawynok, J.; Yaksh, T. L. 1993. Caffeine as an analgesic adjuvant: a review of pharmacology and mechanisms of action. *Pharmacological Reviews* **45**: 43-85.

Sherman, P. M. 1994. The orb-web: an energetic and behavioural estimator of a spider's dynamic foraging and reproductive strategies. *Animal Behaviour* **48**: 19-34.

Shonle, I.; Bergelson, J. 2000. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). *Evolution* **54**: 778-788.

Spronk, F. 1935. Die abhängigkeit der netsbauzeiten der radnetz-spinnen *Epeira diademata* und *Zilla x-notata* von verschiedenen aussenbedingungen. *Zeitschrift für Vergleichende Physiologie.* **C22**: 604-613.

Suter, E. B. 1978. *Cyclosa turbinata* (Araneae, Araneidae): Prey discrimination via web-borne vibrations. *Behavioral Ecology and Sociobiology* **3**: 283-296.

Vollrath, F. 1986. Gravity as an orientation guide during web-construction in the orb spider *Araneus diadematus* (Araneae, Araneidae). *Journal of Comparative Physiology A* **159**: 275-280.

Vollrath, F. 1988. Spiral orientation of *Araneus diadematus* orb webs built during vertical rotation. *Journal of Comparative Physiology A* **162**: 413-419.

Vollrath, F. 1992. Analysis and interpretation of orb spider exploration and web-building behavior. *Advances in the Study of Behavior* **21**: 147-199

Vollrath, F.; Downes, M.; Krackow, S. 1997. Design variability in web geometry of an orb-weaving spider. *Physiology and Behavior* **62**: 735-743.

Vollrath, F.; Mohren, W. 1985. Spiral geometry in the garden spider's orb web. *Naturwissenschaften* **72:**666-667.

Vollrath, F., Nørgaard, T., Krieger, M. Radius orientation in the cross spider *Araneus diadematus*. From *European Arachnology 2000* (S. Toft and N. Scharff eds.) pp 107-116. Aarhus University Press, Aarhus, 2002.

Vollrath, F.; Samu, F. 1997. The effect of starvation on web geometry in an orb-weaving spider. *Bulletin of the British Arachnological Society* **10**: 295-298.

Weissmann, M.; Vollrath, F. 1999. The effect of leg loss on prey capture in *Nephila clavipes*. *Bulletin of the British Arachnological Society* **11**: 198-203.

Witt, P N. 1971. Drugs alter web-building of spiders: A review and evaluation. *Behavioural Science* **16**: 98-112

Witt, P. N.; Rawlings, J. O.; Reed, C. F. 1972. Ontogeny of web-building behavior in two orb-weaving spiders. *American Zoologist* **12**: 445-454.

Witt, P. N.; Reed, C. F. 1965. Spider-web building. *Science* **149**: 1190-1197

Witt P. N.; Reed, C. F.; Peakall, D. B. A spider's web: Problems in regulatory biology. Springer Verlag, Berlin. 1968.

Witt, P. N.; Scarboro, M. B.; Daniels, R.; Peakall, D. B.; Gause, R. L. 1977. Spider web-building in outer space: evaluation of records from the skylab spider experiment. *Journal of Arachnology* **4**: 115-124.

Wolff, D.; Hempel, U. 1951. Versuche über die beeinflussung des netzbaues von *Zilla x-notata* durch pervitin, scopolamin und strychnin. *Zeitschrift für Vergleichende Physiologie* **33**: 497-528.

Zschokke, S. 1993. The influence of the auxiliary spiral on the capture spiral in *Araneus diadematus* Clerk (Araneidae). *Bulletins of the British Arachnological Society* **9**: 169-173.

Zschokke, S. 1994. Web construction behaviour of the orb-weaving spider *Araneus diadematus* Cl. *Ph.D. thesis*. Faculty of Philosophy and Science, University of Basel, Switzerland.

Zschokke, S. 1999. Nomenclature of the orb-web. *The Journal of Arachnology* **27**: 542-546.

Zschokke, S.; Vollrath, F. 1995. Web construction patterns in a range of orb-weaving spiders (Araneae). *European Journal of Entomology* **92**: 523-541.

Zschokke, S.; Vollrath, F. 1995. Unfreezing the behaviour of two orb spiders. *Physiology and Behavior* **58:** 1167-1173

Chapter 2: Prey capture speed of the orb-weaver *Araneus diadematus* Cl. in relation to escape time of prey investigated at different temperatures.

Abstract. The escape time of *Drosophila melanogaster* caught in webs of the orb-weaver *Araneus diadematus* was studied in the laboratory at three different temperatures (14 ˚C, 21 ˚C and 27 ˚C). The results showed that the escape time was significantly faster at higher temperatures. Furthermore, at lower temperatures a significantly higher percentage of fruit flies remained caught in the web for longer than 60 seconds. Reaction time as well as orientation time of the spiders were slower at the lower temperatures leading to a significantly slower overall capture speed at 14 ˚C compared with the higher temperatures. However, the combined temperature effects on the behaviour of the prey and the predator were not additive; correlating capture and escape time gave a theoretical capture success of around 70% at 14 ˚C and 21 ˚C, whereas it was only around 60% at 27 ˚C. This suggests that the cross spider is better at catching fruit flies in the morning or late afternoon than during the day under European high summer temperatures.

Introduction

For spiders, the evolution of silk and its use in webs or as snares has proved tremendously successful. Of the more than 30.000 currently recognised spider species, around 50% are sit-and-wait predators that in one way or another construct silk traps (Foelix 1996). Among the web-building spiders the ecribillate orb weavers are considered to build the most efficient traps (Rypstra 1982). The features of the orb web have evolved to optimise prey capture (Krink and Vollrath 2000; ap Rhisiart and Vollrath 1994; Eberhard 1986; Rypstra 1982). However, there is a considerable variation in web-geometry between individuals and between subsequent webs from the same individual (Sherman 1994; Witt and Reed 1965). The geometry of the orb-web is influenced by a variety of factors, including the spider's age and size (Heiling and Herberstein 1999; Risch 1977; Christiansen et al. 1962; Mayer 1953), the nutritional state (Herberstein et al. 1998; Vollrath and Samu 1997; Sherman 1994), recent prey experiences (Venner et al. 2000; Schneider and Vollrath 1998; Sandoval 1994) and environmental factors (Vollrath et al. 1997). This variation in web design has led to the recognition that the web must be viewed as more than a simple sieve-trap. The web and the spider is now viewed as an integrated system where the web has to perform several different functions in prey capture (Eberhard 1990): Interception of the prey; absorption of the prey's kinetic energy without breaking the web and prevention of escape long enough for the spider to reach the prey.

That the prey's escape time and the spider's capture time have played a major role in the evolution of the orb web can be deduced from the geometry of the web. The majority of vertical orb webs are asymmetrical with most energy being invested in the southern part of the web (Heiling and Herberstein 1999; ap Rhisiart and Vollrath 1994; Vollrath and Mohren 1985). A likely explanation is that the spider, due to gravity, can move faster downward than upward (ap Rhisiart and Vollrath 1994). The likelihood of reaching the prey before it escapes is, therefore, greater in the southern part, making it profitable to invest most time and energy in this part. However, the retention ability of the web is strongly dependent on prey type. Considerable variation in escape time was found for various naturally occurring prey species, with body weight and wing size as important determinants (Nentwig 1982). The most effective way, for a given prey to escape from an orb web, is a high level of activity (Nentwig 1982). Activity in prey insects, as with all poikilothermic animals, is dependent on the ambient temperature (Rott and Ponsonby 2000; Lehmann 1999; Sewell 1979; Precht et al 1973).

However, the prey-capture success does not only depend on the retention ability of the web, but also on the prey catching behaviour of the spider. As spiders are poikilothermic animals, their behaviour is assumed to be temperature-dependent in the same manner as its prey. Model results suggest that ambient temperature is considerably more important than prey density for energy gain of the desert funnel-web spider *Agelenopsis aperta* (Reichert and Tracy 1975). Another indication about the importance of temperature comes from the orb-weaver *Micrathena gracilis*, which orientates its web so as to obtain an optimal body-temperature (Biere and Uetz 1981). Ambient temperature during web-construction is also known to affect the web-geometry, both in the field (Sherman 1994) and in the lab (Vollrath et al. 1997).

Predatory behaviour in the orb-weavers follows a highly stereotyped sequence (Reichert and Luczak 1982), although some interspecific variation is known (Weissmann and Vollrath 1999; ap Rhisiart and Vollrath 1994; Klärner and Barth 1982). Differences are also found depending on the size and potential dangerousness of the prey (Klärner and Barth 1982; Robinson et al. 1969). In this study the fruit fly *Drosophila melanogaster* was used as prey, and various components of the predatory sequence of the cross spider, *Araneus diadematus*, was compared at different temperatures. For each temperature total catching time was coupled to escape time of *Drosophila melanogaster* from empty webs, in order to determine if estimated predatory success varied with temperature.

Materials and methods

Experimental design – Escape time

Orb webs were obtained from juvenile *Araneus diadematus* kept in 30 x 30 x 5 cm frames under standard lab conditions (T = 25 ± 1˚C, 40 ± 5% rH and L/D 16/8). After removal of the spider the intact web was placed in front of an opening cut in a cardboard box (see Fig. 1). Wild type *Drosophila melanogaster* were released in the box and allowed to freely fly around. The animals were taken from a laboratory colony maintained at room temperature (around 20˚ – 22˚C) and reared on Carolina Biological *Drosophila* medium. Some of the flies passed through the opening and became entangled in the web. Intercepted flies were recorded using a surveillance camera connected to a Hi8 Sony Video Recorder allowing frame by frame playback.

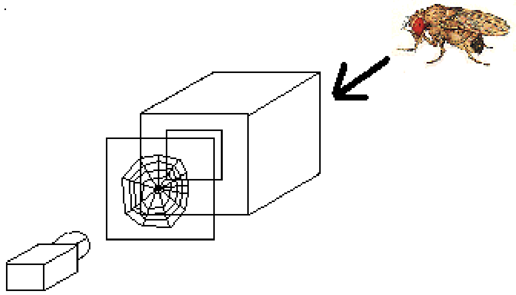


Fig.1. Experimental setup. Flies were placed in the box, which they could only leave by flying out through the window, whereby they got entangled in the web.

Upon contact with the web the flies wriggled vigorously. This activity sometimes caused the thread to break, often resulting in the fly falling down to hit the thread below and so on, causing a slow downward movement of the fly. In these cases the escape time was recorded as the time interval between first and last contact with the web. In a few cases the fly escaped from the web, only later (within a few seconds) to become entangled at a new spot in the web. Here the two capture events were treated as independent, but only the initial entanglement period was recorded.

Flies were recorded as caught in the web, if they touched the web for more than 120 ms. A total of 20 webs, with 4-9 (mean 6.2) flies recorded per web, were used at each temperature. Temperatures used were 14˚C (44% rH), 21˚C (40% rH) and 27˚C (25% rH). The flies used were acclimatised to the given temperature for at least 24 hours prior to the onset of recording. Webs were built within 24 hours of the start of the experiment and were taken directly from the lab.

Experimental design – Capture speed

Juvenile *Araneus diadematus* were collected in the wild during the summer and stored in a refrigerator at a temperature of around 10 °C until used in the experiment. Every third week the spiders were taken out of the fridge, watered and fed 4-6 live fruit flies. Prior to the start of the experiment the spiders were removed from the fridge and put in 30 x 30 x 5 cm plastic frames separated with thin Vaseline smeared plastic sheets. The frames were stacked as books in a bookshelf under standard laboratory conditions (24° ± 2°C, 45% ± 5% rH, 16/8 L/D). During one week of acclimatisation, the spiders were daily given one fruit fly in the webs, whereafter the webs were watered and cut using a hot wire, leaving the silk in the frame for the spider to reingest. On experimental days webs built during the preceding night were transferred in the morning with the spiders in the hub to the climate room. Here they were allowed at least one hour of acclimatisation before the onset of the experiment. The webs were then placed in front of a black and white surveillance camera connected to a Hi8 Sony Video Recorder allowing frame by frame playback. The same prey was used in this experiment as in the one described above. The flies were acclimatised to the climate room for at least 24 hours before being used in experiments. One fruit fly was tossed into the southern quadrant of each web. A soft pincer was used to toss the fly into the web in order to ensure it was alive and wriggling when hitting the web. Each trial was repeated at 3 different temperatures, 14 °C (44% rH), 21 °C (40% rH) and 27 °C (25% rH) with the same spiders. 3-4 days passed between each trial, during which the spiders were returned to standard laboratory conditions and treated the same way as in the acclimatisation period.

Data analysis

For the experiment with escape time each fly was recorded for at least 60 s after being caught by the web. Had the fly not escaped during this time, no escape time was recorded. This kind of data is equivalent to censored data obtained from survival studies. To compare the shape of different survivorship curves several statistical tests are available (Pyke and Thompson 1986). The only difference to the data obtained from this experiment, was that here it was not survivorship curves, but retaining curves, i.e. the proportion of flies caught in the web as a function of time. For this experiment, the non-parametric logrank-test for comparing two or more samples was applied to compare differences in escape time at the different temperatures. A chi-square test was used to test if a difference between temperatures in the proportion of flies with escape times higher than 60 seconds could be found.

From the recordings of predatory behaviour four sets of data were obtained. 1) Distance from the spider to the prey was measured on the monitor using a ruler. 2) Reaction time, i.e. the time elapsed from the prey hitting the web until the spider turned toward it. 3) Orientation time, i.e. the time elapsed from the spider having turned toward the prey until it left the hub. 4) Capture time, i.e. the time elapsed from the spider leaving the hub until it touched the prey with the chelicerae. Capture speed was calculated by assuming a linear relationship between capture time and distance. In a few cases the spider showed no sign of having detected the fly, or turned toward it, but did not rush out to catch it. In some cases the spider would eventually after several minutes catch the prey, but in other cases the prey was still untouched in the web when recording was terminated. A cut-off point of 10 seconds was from these observations decided upon. Meaning that if the spider’s turning or reaction time was longer than 10 sec, the recording was excluded from the analysis. In order to approach normality and acquire equal variances all time data were transformed using the Box-Cox power-transformation. Capture speed was transformed using the natural logarithm. Transformed data were analysed using a multivariate analysis of variances (MANOVA) for repeated measures. The Pillai-Bartlett trace criterion was used, because of its robustness to assumption violations (Keselman 1998). Correlations with distance were investigated using the Pearson's correlation coefficient. All statistical tests were performed using the software JMP 3.2.2 (Macintosh version, SAS Institute Inc. 1997) with the significance level set at $\alpha = 0.05$.

Results

Out of the 36 spiders used for the prey escape experiment, 30 built webs consistently enough to contribute with one or more webs. As the 20 webs used at each temperature were obtained from different individuals, each temperature trial used webs from a distinct group of spiders. However, 8 spiders contributed with webs at all three temperatures.

Each fly caught in the web resulted in a minor disruption in the web on the spot where it was seized by the spider. It was, therefore, to be expected that as more flies hit the web it would be progressively easier to escape from it. However, no such trend was found for the first 6 flies (flies 7-9 excluded due to few data) caught in each web (14˚C: Log-rank $\chi^2 = 1.67$, df = 5, P = 0.89; 21˚C: Log-rank $\chi^2 = 2.13$, df = 5, P = 0.83; 27˚C: Log-rank $\chi^2 = 2.78$, df = 5, P = 0.73).

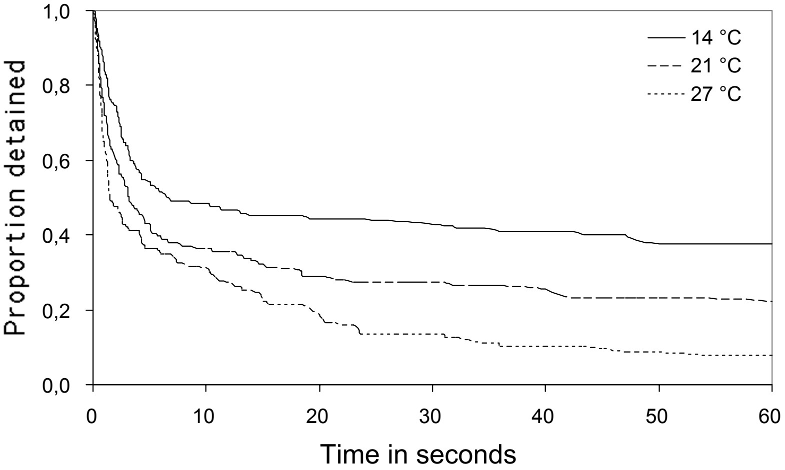


Fig. 2. The proportion of intercepted flies detained in the web as a function of time. Each curve represents a different temperature.

There was a difference in the shape of the detaining curves, with the slowest rate of escape from the web at low temperature and progressively higher escape rates at increasing temperatures (see Fig. 2). A statistical analysis revealed that there was a significant difference between the shapes of the curves (Logrank $\chi^2 = 30.17$; df = 2, P < 0.0001), indicating that it took longer for the flies to free themselves from the web at low temperatures than at high temperatures. Furthermore, more flies managed to escape from the web within 60 sec. as temperature increased (see Table 1).

Table 1: The effect of temperature on the number of escaped flies

	Temperature			Chi-square statistics		
	14˚C	21˚C	27˚C	χ^2	df	P
Total number of flies	122	121	126			
Not escaped after 60 s.	46 (37.7%)	27 (22.3%)	10 (7.9%)	24.3	2	< 0.001

The second row is the proportion of flies that had not escaped 60 s after hitting the web. The standard chi-square test was used to compare the proportions across the three temperatures.

In the experiment with predatory behaviour, the initial distance between the spider and the fruit fly was intended to be constant. However, although care was taken when tossing flies at the webs, exact placement was not possible. This together with differences in web-size resulted in variable distances from spider to prey for each sample. The mean distance (± std. dev.) at 14°C was 62 ± 18 mm, at 21 °C 65 ± 16 mm and at 27 °C it was 54 ± 14 mm. There was neither correlation between distance and reaction time for any of the three temperatures (T = 14°C, $R^2 = 0.29$, p = 0.20; T = 21°C, $R^2 = 0.12$, p = 0.61; T = 27°C, $R^2 = -0.01$, p = 0.95) nor between distance and orientation time (T = 14°C, $R^2 = 0.16$, p = 0.49; T = 21°C, $R^2 = 0.10$, p = 0.67; T = 27°C, $R^2 = 0.15$, p = 0.51). A linear regression analysis was carried out to test the assumption of a linear relationship between distance and capture time. The analysis gave a significant result only for the low temperature (T = 14°C; F = 5.1, df = 19, p = 0.04. T = 21°C; F = 2.3, df = 19, p = 0.14. T = 27°C; F = 3.1, df = 19, p = 0.09). However, considering that all F-values are relatively high and the number of replicas is relatively low, it is justifiable to proceed with calculating the capture speed assuming a linear relationship between distance and capture time for all three temperatures.

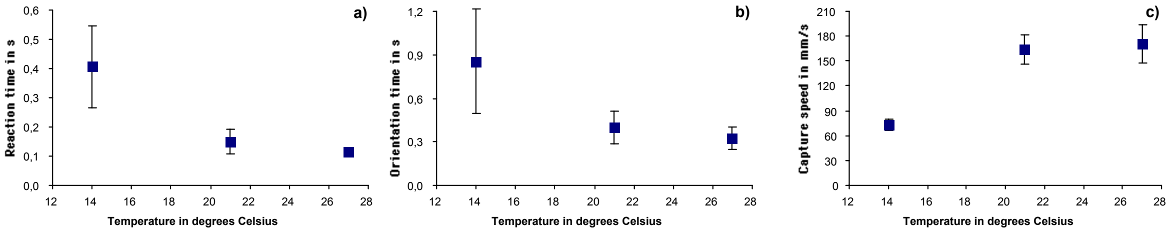


Fig. 3. Predatory behaviour of *Aranues diadematus* preying on *Drosophila melanogaster* at different temperatures. Each data point is the based on 21 predatory events. a) Mean reaction time in seconds at temperatures of 14°C, 21°C and 27°C. b) Mean orientation time in seconds at temperatures of 14°C, 21°C and 27°C. c) Capture speed in millimetre per second at temperatures of 14°C, 21°C and 27°C. Error bars indicate standard error of the mean.

The reaction time of *Araneus diadematus* to incoming prey was significantly different for the three temperatures tested (MANOVA $F_{2,19} = 6.96$, p = 0.0054). From Figure 3a it is evident that the reaction time at the low temperature was significantly longer than at the two higher temperatures. The time interval between impact of the fly and the first reaction of the spider was more than twice as long at 14°C. The same trend was found for orientation time (see Fig. 3b), although no significant differences were found (MANOVA $F_{2,19} = 3.10$, p = 0.068). But similar to reaction time, the orientation time was more than twice as long at 14°C compared to 21°C and 27°C. As shown in

Figure 3c capture speed increased with temperature (MANOVA $F_{2,19} = 20.74$, $p < 0.0001$). The speed at which the spider rushed out to seize the prey was more than two times slower at 14°C compared with the higher temperatures. However, Figure 3 indicates that the observed effect of temperature was not additive. The difference between the two highest temperatures was insignificant, although slightly faster at 27 °C for all 3 aspects of predatory behaviour tested here.

When bringing together the results of the experiment with escape time and the experiment with capture speed, the capture efficiency can be estimated for the temperatures tested. The proportion of fruit flies caught was calculated to be around 70% for the two lowest temperatures, whereas it dropped to 61% for the highest temperature (see table 2).

Table 2. Total catching time and the corresponding capture success for *Araneus diadematus* preying on intercepted *Drosophila melanogaster*.

Temperature	*Catching time*	*Conf. interval*	*Prop. caught*	*Conf. Interval**
14 °C	*2.38 s*	*1.29 s – 3.47 s*	*69%*	*60% - 82%*
21 °C	*1.20 s*	*0.74 s – 1.66 s*	*71%*	*63% - 78%*
27 °C	*1.14 s*	*0.72 s – 1.56 s*	*61%*	*50% - 75%*

Catching time is calculated from capture speed, with a constant distance set at 60 mm, and added to reaction time and orientation time for each of the 21 samples at each temperature. The next column gives the 95% confidence interval on catching time. The proportion caught is the percentage of flies still detained in the web, at the given temperature, when the catching time has elapsed. These values were read from figure 2. The last column gives the confidence interval of the proportion caught. This was found by reading the values of the confidence interval of the catching time from figure 2.

Discussion

In Denmark, the cross spider is active late April to October (pers. obs.). During this period it encounters a wide range of temperatures. The following data was obtained from the Danish Meteorological Institute. In April the average day temperature is 9.6 °C (max temperature recorded for 2002 was 23°C) and the average night temperature is 2.1 °C (min temperature recorded for 2002 was –5 °C). In August the average day temperature is 20.0 °C (max temperature recorded for 2002 was 32 °C) and the average night temperature is 11.3 °C (min temperature recorded for 2002 was 8 °C). The results of this study showed that temperature had a significant impact on the dynamics of the predator-prey system in orb-weavers. There was an effect of temperature on both the escape time of prey from an orb web and on the orb-weavers reactions towards prey, with increasing temperatures resulting in faster dynamics. But what possible mechanisms could lie behind this?

Contrary to cribellate orb webs, the retention power of webs built by ecribellate orb-weavers such as *Araneus diadematus* depends on the stickiness of the individual capture thread (Vollrath 1992). The capture threads have a hygroscopic coating, which gives them their elasticity (Foelix 1996). The water held by the coat forms droplets, interspaced on the threads and inside these are small nodules consisting of gluey material. Thus the adhesiveness of the web depends on the air's relative humidity (Edmonds and Vollrath 1992). To my knowledge there is no published work on any effects of temperature on the adhesiveness of the capture thread. However, even if such an effect should exist, it is unlikely to play any major role. Edmonds and Vollrath (1992) found only a 20% increase in droplet volume when raising the relative humidity from 45% to 84%. Since the numerical difference in relative humidity in the present study is only 20%, it must be considered unlikely that this can explain more than a small fraction of the observed differences in escape time. Variation in relative humidity at the different temperatures could partly explain the results, since humidity is known to affect the geometry of the web (Vollrath et al. 1997). However, the webs used here were all built under standard laboratory conditions, and were only brought to the climate room at the start of the experiment. Lastly, the difference in relative humidity presumably has no influence on the behaviour of the fruit flies, since short-term fluctuations in humidity seem to have little effect on insect activity (Morandin et el. 2001; Rott and Ponsonby 2000; Wright and Morton 1995).

The geometry of the web might have had an effect on the retention power. A possible source for this may be the mesh size, i.e. the distance between subsequent turns of the capture spiral. With a smaller distance the chance that prey become entangled by more than one thread increases and the more threads that contact the prey the longer the prey will be detained (Eberhard 1986). However, this possibility can be excluded as having an impact on the results of this study, since the webs used at the different temperatures were selected at random, so any directional effect of web geometry must be considered negligible.

The discussion above leads us to the conclusion that the effects of temperature on escape time found in this study primarily must be due to its impact on the prey. This is in accordance with other studies on the relation between temperature and activity-level in *Drosophila melanogaster* (Lehmann 1999; Sewell 1979). Muscle mechanical power in *Drosophila melanogaster* increases with increasing temperatures from 5 °C to 30 °C (Lehmann 1999). Thus the results of this study indicate a support to Nentwig's (1982) hypothesis that an increase in activity (i.e. wriggling) of captured prey results in a decrease in escape time.

The second part of this study suggests that the spider's predatory behaviour is also influenced by ambient temperature, with low temperatures resulting in slower detection of and approach to prey. Again differences in humidity at the three temperatures tested, could influence the results obtained. However, as discussed above humidity is unlikely to have any impact on either predator or prey behaviour. The spider detects and localises the intercepted prey through thread-transmission of vibrations generated by the struggling prey (Landolfa and Barth 1996; Masters et al. 1986). Since the biomechanical properties of silk are affected by wetting (Work 1981), it is possible that changes in relative humidity may affect the transmission of vibrations from prey to spider. However, even if such an effect were present, its impact would be expected to be negligible considering the temporal resolution used in this study. Another important aspect of predatory behaviour is the nutritional state of the spider. Food deprived orb-weavers have a shorter catching time than food satiated spiders (Herberstein et al.1998). In the present study, spiders were on a strict diet comprising of one fruit fly per web built. Although this is a standard laboratory feeding regime, it is possible that it would result in starvation of the spiders on longer time scales. Spiders are, however, very resistant to starvation (Foelix 1996) and the spiders used here showed no sign of malnutrition (i.e. a deflated abdomen) after the three weeks the experiment lasted. To calculate capture speed a linear relationship between capture time and distance was postulated. The statistical analysis supports this assumption. However, it is likely that long distances result in a slower average speed, since spiders are known to make reorientation stops en route to prey (Weissmann and

Vollrath 1999; Suter 1978). Presumably reorientation stops are more frequent with longer distances to the prey. More research on this issue is necessary to deduce a more precise relationship between distance and capture speed. The distances used in this study, however, were relatively short and the assumption of linearity therefore justifiable.

The results of predatory behaviour obtained in this study agree with earlier studies. None of the earlier studies, however, reported under which temperature their experiments were conducted, but presumably room temperature was used. Ap Rhisiart and Vollrath (1994) report that immature *Araneus diadematus* preying on *Locusta migratoria* (length ≈ 10 mm) show a reaction time of 0,41 ± 0,36 s, an orientation time of 0,49 ± 0,40 s and a capture speed of 164,4 ± 14,5 mm/s. This agrees very well with the results presented here, except that their reaction time is twice as long. This can probably be explained by the different prey species used or the different way of tossing the prey into the web. Detection of prey depends on the initial activity of the prey (Suter 1978), which again is species dependent (Nentwig 1982). In the study presented here, care was taken to handle the fruit flies carefully to ensure that they actively tried to free themselves upon entanglement.

This study indicates that there is a pronounced effect of temperature on both the prey's escape time and *Araneus diadematus*' catching time. However, whereas an additive effect was found for escape time, with increasing temperatures leading to correspondingly faster escape time, the effect on the spider seemed to be non-additive in such a way that increases in temperature only resulted in faster catching times in the lower temperature range. With a further increase in temperature the catching time levelled off, which resulted in a slightly higher capture efficiency at the two lower temperatures. This suggest that there is an optimal temperature for catching fruit flies, which is lower than the temperature encountered on warm summer days even in temperate climates. On these days *Araneus diadematus* is expected to have a higher success rate when capturing prey in early morning or late afternoon. However, further studies, using other types of prey, are needed before any general conclusions can be made. Escape time depends on type and activity level of the prey (Nentwig 1982). Activity level depends on body temperature, which again depends on insect size (Lactin and Johnson 1998). It is, therefore, possible that other prey species will be affected by temperature in a different manner than *Drosophila melanogaster*.

In the experiments conducted here, the predator-prey dynamics were investigated at different temperatures. However, one aspect of the predator-prey system was ignored. The geometry of the web, and especially mesh size, determines retention abilities for different prey size (Schneider and Vollrath 1998; Uetz et al. 1978). Since temperature is known to affect web-geometry (Vollrath et al. 1997), further studies are needed to investigate the influence of web-geometry on the temperature-dependent predator-prey dynamics described here.

References

Ap Rhisiart, A.; Vollrath, F. 1994. Design features of the orb web of the spider, *Araneus diadematus*. *Behavioral Ecology* **5:**280-287.

Biere, J. M.; Uetz, G. W. 1981. Web orientation in the spider *Micrathena gracilis* (Araneae: Araneidae). *Ecology* **62**: 336-344.

Christiansen, A.; Baum, R.; Witt, P. N. 1962. Changes in webs brought about by mescaline, psilocybin and an increase in body weight. *J. Pharmac. Exp. Ther.* **136**: 31-37.

Eberhard, W. G. 1986. Effects of orb-web geometry on prey interception and retention. From William A. Shear (ed.) *Spiders: Webs, behaviour and evolution.* p. 70-100.

Eberhard, W. G. 1990. Function and phylogeny of spider webs. *Annual Review of Ecology and Systematics* **21**: 341-372.

Edmonds, D. T.; Vollrath, F. 1992. The contribution of atmospheric water vapour to the formation and efficiency of a spider's capture web. *Proceedings of the Royal Society of London. Series B.* **248:** 145-148.

Foelix, R. F. *Biology of spiders 2nd Ed.* Oxford University Press, Oxford. 1996

Heiling, A. M.; Herberstein, M. E. 1999. The role of experience in web-building spiders (Araneidae). *Animal Cognition* **2**: 171-177.

Herberstein, M. E.; Abernethy, K. E.; Backhouse, K.; Bradford, H.; de Crespigny, F. E.; Luckock, P. R.; Elgar, M. A. 1998. The effect of feeding history on prey capture behaviour in the orb-web spider A*rgiope keyserlingi* Karsch (Araneae: Araneidae). *Ethology* **104**: 565-571.

Keselman, H. J. 1998. Testing treatment effects in repeated measures designs: an update for psychophysiological researchers. *Psychophysiology* **35**: 470-478.

Klärner, D.; Barth, F. G. 1982. Vibratory signals and prey capture in orb-weaving spiders (*Zygiella x-notata*, *Nephila clavipes*; Araneidae). *Journal of Comparative Physiology A* **148**: 445-455.

Krink, T.; Vollrath, F. 2000. Optimal area use in orb webs of the spider *Araneus diadematus*. *Naturwissenschaften* **87**: 90-93.

Lactin, D. J.; Johnson, D. L. 1998. Convective heat loss and change in body temperature of grasshopper and locust nymphs: Relative importance of wind speed, insect size and insect orientation. *Journal of Thermal Biology* **23**: 5-13.

Landolfa, M. A.; Barth, F. G. 1996. Vibrations in the orb web of the spider *Nephila clavipes*: cues for discrimination and orientation. *Journal of Comparative Physiology A*. **179**: 493-508.

Lehmann, F. O. 1999. Ambient temperature affects free-flight performance in the fruit fly *Drosophila melanogaster*. *Journal of Comparative Physiology B*. **169**: 165-171.

Masters, W.M.; Markl, H. S.; Moffat, A. J. M. Transmission of vibration in a spider's web. From *Spiders: webs, behavior and evolution*. Ed. Shear, W. A. Stanford University Press. Stanford. 1986.

Mayer, G. 1953. Untersuchungen über herstellung und struktur des radnetzes von *Aranea diadema* und *Zilla x-notata* mit besonderer berücksichtigung des unterscheides von jugend- und altersnetzen. *Zeitschrift für Tierpsychologie* **9**: 337-362.

Morandin, L. A.; Laverty, T. M.; Kevan, P. G.; Khosla, S.; Shipp, L. 2001. Bumble bee (Hymenoptera: Apidae) activity and loss in commercial greenhouses. *Canadian Entomologist* **133**: 883-893.

Nentwig, W. 1982. Why do only certain insects escape from a spider's web? *Oecologia* **53**: 412-417.

Pyke, D. A.; Thompson, J. N. 1986. Statistical analysis of survival and removal rate experiments. *Ecology* **67**: 240-245.

Precht, H.; Christophersen, J.; Hensel, H.; Larcher, W. *Temperature and life*. Springer-

Verlag Berlin Heidelberg New York 1973.

Reichert, S. E.; Luczak, J. Spider foraging: behavioral responses to prey. From *Spider communication mechanisms and ecological significance*. Eds. Witt, P.N and Rovner, J. S. Princeton University Press. 1982.

Reichert, S. E.; Tracy, R. 1975. Thermal balance and prey availability: bases for a model relating web-site characteristics to spider reproductive success. *Ecology* **56**: 265-284.

Risch, P. 1977. Quantitative analysis of orb web patterns in four species of spiders. *Behavior Genetics* **7**: 199-238.

Robinson, M. H.; Mirick, H.; Turner, O. 1969. The predatory behavior of some araneid spiders and the origin of immobilization wrapping. *Psyche* **70**: 487-501.

Rott, A. S.; Ponsonby, D. J. 2000. The effects of temperature, relative humidity and host plant on the behaviour of *Stethorus punctillum* as a predator of the two-spotted spider mite, *Tetranychus urticae*. *BioControl* **45**: 155-164.

Rypstra, A. L. 1982. Building a better insect trap; an experimental investigation of prey capture in a variety of spider webs. *Oecologia* **52**: 31-36.

Sandoval, C. P. 1994. Plasticity in web design in the spider *Parawixia bistriata*: a response to variable prey type. *Functional Ecology* **8**: 701-707.

Schneider, J. M.; Vollrath, F. 1998. The effect of prey type on the geometry of the capture web of *Araneus diadematus*. *Naturwissenschaften* **85**: 391-394.

Sewell, D. F. 1979. Effect of temperature and density variation on locomotor activity in *Drosophila melanogaster*: a comparison of behavioural measures. *Animal Behaviour* **27**: 312-313.

Sherman, P. M. 1994. The orb-web: an energetic and behavioural estimator of a spider's dynamic foraging and reproductive strategies. *Animal Behaviour* **48**: 19-34.

Suter, E. B. 1978. *Cyclosa turbinata* (Araneae, Araneidae): Prey discrimination via web-borne vibrations. *Behavioral Ecology and Sociobiology* **3**: 283-296.

Uetz, G. W.; Johnson, A. D.; Schemske, D. W. 1978. Web placement, web structure, and prey capture in orb-weaving spiders. *Bulletin of the British Arachnological Society*. **4**: 141-148.

Venner, S.; Pasquet, A.; Leborgne, R. 2000. Web-building behaviour in the orb-weaving spider *Zygiella x-notata*: influence of experience. *Animal Behaviour* **59**: 603-611.

Vollrath, F. 1992. Analysis and interpretation of orb spider exploration and web-building behavior. *Advances in the Study of Behavior* **21**: 147-199.

Vollrath, F.; Downes, M.; Krackow, S. 1997. Design variability in web geometry of an orb-weaving spider. *Physiology and Behavior* **62**: 735-743.

Vollrath, F.; Mohren, W. 1985. Spiral geometry in the garden spider's orb web. *Naturwissenschaften* **72:**666-667.

Vollrath, F.; Samu, F. 1997. The effect of starvation on web geometry in an orb-weaving spider. *Bulletin of the British Arachnological Society* **10**: 295-298.

Weissmann, M.; Vollrath, F. 1999. The effect of leg loss on prey capture in *Nephila clavipes*. *Bulletin of the British Arachnological Society* **11**: 198-203.

Witt, P. N.; Reed, C. F. 1965. Spider web-building. *Science* **149**: 1190-1197.

Work, R. W. 1981. A Comparative study of the super-contraction of major ampullate silk fibers of orb-web-building spiders (Araneae). *Journal of Arachnology* **9**: 299-308.

Wright E. J.; Morton, R. 1995. Daily flight activity of *Trogoderma variabile* (Coleoptera, Dermestidae) and *Rhyzopertha dominica* (Coleoptera, Biostrichidae). *Journal of Stored Products Research* **31**: 177-184.

Chapter 3: Web-building behaviour and web-geometry of the orb-weaver *Araneus diadematus* Cl. at different temperatures

Abstract: The effect of temperature on the web-building behaviour and the resultant orb web was studied using the orb-weaver *Araneus diadematus* as a model organism. Temperature had no effect on any of the web-parameters examined, but had significant impacts on total building time and speed during web construction. Furthermore, an increase in the pause between construction of the auxiliary and capture spiral was observed. The results indicate that the orb web show no adaptation to temperature nor does the spider compensate, by constructing smaller webs, for the longer time needed to build a web at lower temperatures.

Temperature plays an important role in the lives of most organisms (Precht et al. 1973). Especially ectotherm animals rely on adequate temperatures to maintain a sufficient activity level for displaying complicated behaviour (Laudien 1973). The orb-webs of the cross spider, *Araneus diadematus*, are common sights in most open land in the northern temperate region. In Denmark the cross spider is active from early May to late October. In this period it encounters a wide range of ambient temperatures. Web-building predominantly occurs at darkness in the early morning (Ramousse and Davis 1976), but the precise onset of building is strongly influenced by ambient temperature (Spronk 1935). Temperature also affects other aspects of web-building behaviour. The forest-dwelling orb-weaver *Micrathena gracilis* orientates it web in such a direction that solar radiation provides an optimal body-temperature for most of the day (Biere and Uetz 1981). Furthermore, given a choice of temperatures the tangle-web builder *Achaearaneae tepidariorum* chooses an optimal temperature for web-construction at which the webs produced are more efficient at capturing prey (Barghusen et al. 1997).

The highly structured two-dimensional orb-web is easily photographed and analysed for multiple geometric properties, and has therefore been used extensively for invertebrate behavioural studies (see Vollrath 1992 for a review). The detailed geometry of the web is highly sensitive to internal factors in the spider, such as age and size (Heiling and Herberstein 1999; Risch 1997; Christensen et al. 1962; Mayer 1953), nutritional state (Herberstein et al. 1998; Vollrath and Samu 1997; Sherman 1994) and recent prey experiences (Venner et al. 2000; Schneider and Vollrath 1998; Sandoval

1994). However, external factors such as, physical surroundings at the web-site (Krink and Vollrath 2000; Vollrath et al. 1997) and wind (Vollrath et al. 1997; Hieber 1984), influence the geometry. Considering the variety of factors influencing the orb-web, it is to be expected that temperature also has an effect on the geometry of the web.

Vollrath and co-workers (1997) looked at the effect of decreasing the temperature from 24 °C to 12 °C and then again increasing the temperature to 24 °C. They observed a decrease in spiral length, in number of reverses and in spiral turns and an increase in mesh size. Most of these changes were restored to initial values when the temperature again was raised to 24 °C. A possible explanation for these changes could be that the spider adapts the web-geometry to the prevailing conditions. At colder temperatures most small insects decrease their flying activity (Lehmann 1999; Fredeen and Mason 1991; Williams and Osman 1960), whereas larger insects, such as the near endothermic honey bees and bumblebees, are expected to continue flying at much lower temperatures. Efficiency of retaining different prey sizes in the orb web depends on the mesh size (Eberhard 1986; Uetz et al. 1978), so the increased mesh size observed by Vollrath and co-workers (1997) could be an adaptation to catch larger insects. This hypothesis is supported by results indicating that the size of previous captured prey affects mesh size in future webs (Schneider and Vollrath 1998; Sandoval 1994). An alternative explanation for their observations could be that the spider's speed of web-building is lower at lower temperatures and that the spider compensates for the increased web-building time by laying down fewer capture spiral threads. Construction of the capture spiral takes up about 80% of total web-building time (Foelix 1996), so laying down fewer capture spiral threads can save considerable time. A possible reason for minimising web-building time is that the spider faces a larger predation risk during web-construction compared with sitting motionless in the hub or the retreat.

This study attempts to distinguish between the two hypotheses by examining the web-geometry at three different temperatures and relate these findings to observations of web-building behaviour and speed at two different temperatures employing computer-automated video tracking. This technique is useful for quantitatively analysing locomotory behaviour in invertebrates (Baatrup and Bayley 1993) and has also been successfully applied for analysing web-building behaviour in orb-weavers (Zschokke and Vollrath 1995a,b).

Materials and methods

Experimental design:

Juvenile orb-weavers, *Araneus diadematus,* were exposed to temperatures of 14 °C, 20 °C and 26 °C and the constructed webs analysed. Juvenile spiders were caught at Mols in Eastern Jutland, Denmark and placed in the fridge at 10 °C until start of the experiment. Spiders were then put into a climate cabinet (Termaks type KBP 6395 KL) and housed individually in 30 x 30 x 5 cm Perspex frames separated by thin Vaseline smeared plastic sheets. Approximately half of the spiders were first introduced to 14 °C and the rest first to 26 °C and allowed 3-5 days of acclimatisation before any webs were obtained. Spiders were either subjected to an increasing (14 °C → 20 °C → 26 °C) or decreasing (26 °C → 20 °C → 14 °C) temperature sequence and only spiders that build webs at each temperature were included in the analysis. The climate cabinet was set to a daily cycle of 16 hours light and 8 hours darkness with a constant humidity at 45% rH. The 3rd or 4th web built at each temperature was placed in the opening of a back-lit box and photographed with a Nikon Coolpix 995 digital. The experiment ran for a maximum of 4 weeks at each trial, during which time the spiders were watered daily and fed one fruit fly per web constructed. Webs were cut using a hot wire and leaving the frame and one radius in the north and south quadrant intact, thereby collapsing the capture spiral into one single thread, which was left in the frame for the spider to ingest.

Another set of juvenile *Araneus diadematus* was used for recording of web-building behaviour at 15 °C and 23°C. Spiders were housed individually in 20 x 20 x 5 frames in the climate cabinet. Again approximately half of the spiders were first introduced to the low temperature and the rest first to the high temperature and allowed minimum one week of acclimatisation to the climate cabinet. The conditions in the climate cabinet were the same as in the previous experiments, except that constant light was required for continuous recording. Darkness was simulated by covering the light in the climate cabinet with red cellophane, as spiders are unable to see red light (Baatrup and Bayley 1993). An extra light source was introduced to the climate room to obtain the 16/8 L/D cycle. The experimental setup for recording the behaviour is shown in Figure 1. Two black and white surveillance cameras were fitted in the climate cabinet and recorded the movement in the frames via a mirror. They were connected to two 'VP112' scanning units (or Baker boxes) that again were connected to a Macintosh Performa 460 computer. The Baker box scanned the image recorded by the surveillance camera to continuously monitor the position of the spider. Every new position was transferred to the computer running the application Move Recorder for the Macintosh (Zschokke 1994), which stored the positions as x-y co-ordinates.

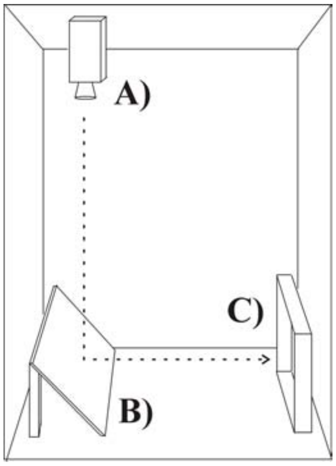


Fig. 1. The experimental set-up. A look into the climate cabinet. The surveillance camera (A) was mounted in a vertical position and recorded the movements of the spider in the frame (C) via a mirror (B). Not shown on this figure are two frames and sheets on each side of C) and the second camera and frame placed parallel to the ones showed.

Data analysis:

The digital images were converted to pixel-graphic and analysed using the software WebExtractor (Pedersen 1995). Additional measurements were obtained with a ruler from printouts of the images. From each web the following data were extracted and analysed.

- Radii length: Total length of all radii.
- Capture length: Total length of all capture spiral threads.
- Capture area. The area of the capture area excluding the hub and free zone.

- No. of reverses per cm spiral thread. During capture spiral-construction the spider reverses its direction of thread laying several times, most often in the southern quadrant where more spiral turns are laid. Since the number of reverses presumably depends on the size of the web, it was here divided by capture length.
- Proportion of irregular radii. The number of Y-shaped (radii that splits into two inside the capture area) and distorted radii as a percentage of total number of radii. The degree of distortion was estimated visually from the printout and the radii was said to be distorted if it deviated with more than around 5 degrees from a straight line.
- Mesh size: Average mesh size was calculated by inserting a vertical line through the hub centre and employing the following formula derived by Herberstein and Tso (2000).

  $$\frac{1}{2}\left(\frac{r_u - Hr_u}{S_u - 1} + \frac{r_l - Hr_l}{S_l - 1}\right)$$

  Where r_u and r_l are the upper and lower vertical capture spiral lengths. Hr_u and Hr_l are the upper and lower vertical lengths of the hub and the free zone (i.e. the area between the hub and the capture spiral) taken together. In this study the centre of the web was defined as the midpoint of the vertical line through the free zone and the hub, thereby keeping Hr_u and Hr_l of equal lengths. S_u and S_l are the number of capture spiral turns in the upper and lower part respectively.
- Eccentricity: A measure of the shape of the web, calculated by using the following formula (Vollrath et al. 1997).

  $$\sqrt{1 - \left(\frac{L_{hor}}{L_{vert}}\right)^2}$$

  Where L_{hor} is the width and L_{vert} the height of the capture spiral. If the width was longer than the height, the term in the brackets was inverted and eccentricity assigned a negative value.

From the recordings of movements during web-building the following measurements were obtained and analysed.

- Total building time. The total time elapsed from the spider creating the first radii until finishing the last capture spiral. Pauses during which the spider sits motionless is included in this time.
- Speed – auxiliary spiral. The average building speed of the spider when constructing the auxiliary spiral. Pauses were not included in this measurement.
- Speed – capture spiral. The average building speed of the spider when constructing the capture spiral. Pauses were not included in this measurement.
- Pause from aux – cap. The pause between finishing the auxiliary spiral and starting on the first capture spiral turn. This pause is seen in all orb-weavers (Foelix 1996) and could be a necessary break used to change from normal silk to gluey silk.

The web-parameters obtained from the web-photographs were compared between the three temperatures by applying a multivariate test of variance (MANOVA) for repeated measures. The Pillai-Bartlett test criterion was used, because of its robustness to assumption violations (Keselman 1998). For comparison between the two temperatures used in the web-building behaviour experiment, a two-tailed paired t-test was used. Since several tests were performed on the same data-set, a sequential Bonferroni adjustment was made to minimise type I errors (Rice 1989). The initial significance level was set at 5%. Statistical tests were performed using JMP 3.2.2 for the Macintosh (SAS Institute Inc. 1997) and SPSS 10.0 (SPSS Inc. 1999).

Results

The environment in the climate cabinet had a severe negative effect on web-building frequency. Out of the 64 spiders placed in the climate cabinet for the web-geometry experiment only 13 spiders built three or more consecutive webs at each of the three temperatures. Similar with the web-building experiment where only 5 out of 48 spiders built sufficiently to be included in the analysis.

Table 1. The effects of temperature on web-geometry

	14 °C	20 °C	26 °C	$F_{2,11}$*	P		Adjusted α#
Number of spiders	13	13	13				
Radii length/mm	2864 ± 180	2744 ± 151	2704 ± 169	0.56	0.589	>	0.013
Capture spiral length/mm	10539 ± 833	9953 ± 604	9729 ± 824	0.44	0.655	>	0.025
Capture area/mm^2	24911 ± 2373	24556 ± 1433	23666 ± 2291	0.14	0.868	>	0.050
Irregular radii in %	29.2 ± 2.9	24.2 ± 2.2	26.4 ± 3.4	1.71	0.226	>	0.007
Reverses per m spiral thread	0.87 ± 0.07	1.04 ± 0.17	0.97 ± 0.16	0.55	0.594	>	0.017
Mesh size in mm	2.46 ± 0.12	2.54 ± 0.10	2.56 ± 0.12	0.91	0.432	>	0.008
Eccentricity	0.49 ± 0.07	0.51 ± 0.03	0.55 ± 0.04	0.62	0.557	>	0.010

All values are given with the standard error of the mean.
* Measurements were compared using the MANOVA for repeated measures with the Pillai-Bartlett trace criterion. There were 2 between-subject degrees of freedom and 11 within-subject degrees of freedom.
\# Since several tests were performed on the same data-set, the significance levels were adjusted using the sequential Bonferroni method.

As can be seen from Table 1 there were no effects of temperature on any of the web-parameters investigated in this study. There was a very slight tendency of a decrease in general web-size and an increase in mesh size, eccentricity and in the number of reverses with an increase in temperature, but this was too vague to allow for any conclusions being made. Furthermore, there was an indication that radial irregularity might be increasing at the extreme temperatures.

Table 2. The effects of temperature on web-building behaviour

	15 °C	23 °C	t_4*	P		Adjusted α#
Number of spiders	5					
Total building time / min.	78.1 ± 6.4	43.3 ± 2.0	5.58	0.0050	<	0.025
Speed – auxiliary spiral / mm/s	4.7 ± 0.5	7.8 ± 0.6	-6.07	0.0037	<	0.017
Speed – capture spiral / mm/s	3.5 ± 0.2	5.9 ± 0.3	-15.18	0.0001	<	0.013
Pause from aux to cap / s	38 ± 7	18 ± 2	2.77[a]	0.0501	>	0.050

All values are given with the standard error of the mean.
* Measurements were compared using the two tailed paired t-test.
\# Since several tests were performed on the same data-set, the significance levels were adjusted using the sequential Bonferroni method.
a. Data was transformed with the natural logarithm to acquire homogeneity of variances.

The web-building behaviour showed clear effects of temperature (Table 2). Total time needed for construction of the orb web was nearly halved from 15 °C to 23 °C. Speed of construction for both the auxiliary and the capture spiral significantly increased at the higher temperature. The break between termination of the auxiliary spiral and continuation of the capture spiral was similarly reduced from 38 s at 15 °C to 18 s at 23 °C, however this was marginally non-significant.

Discussion

The cross spider *Araneus diadematus* lives in temperate climates and therefore experiences both daily and seasonal thermal gradients. The orb spider is an ectotherm animal so ambient temperature has an important influence on most aspects of its behaviour (Precht et al. 1973). Surprisingly, the results of the present study suggest that temperature had no effect on web-geometry. However, now firm conclusions concerning this can be drawn from the data, as the sample size was too low due to the spiders' reluctance to build webs in the climate cabinet. The reason for this observed effect on web-building frequency is not clear. The lighting regime and humidity in the climate cabinet were equal to standard lab conditions where this reluctance to build web was not observed. A possible explanation could be that the airflow or vibrations generated by the cooler in the climate cabinet disturbed the spiders. Furthermore there could be a different barometric pressure in the climate cabinet, which might conceivably influence the web-building frequency.

The results of this study do not in agree with a very similar study conducted by Vollrath and co-workers (1997). They found that a drop in temperature from 24 °C to 12 °C resulted in a significant decrease in spiral length and number of reverses, as well as an increase in mesh size. Whereas the data presented here showed a slight tendency for an overall decrease in web-size with lower temperatures, there was no tendency to an increase in mesh size at low temperatures, on the contrary there seemed to be a minor decrease. Except from a somewhat higher humidity (55% rH vs. 45% rH) and a lower temperature (12 °C vs. 14 °C) the experimental conditions used by Vollrath and co-workers (1997) were the same as the ones used in the present study. A lower testing temperature would have been interesting, however, it was feared that this would result in an even lower web-building frequency than the one already observed. In both studies the acclimatisation period was quite short with only 3-4 days before the first webs were obtained and this might explain the differences observed. It is possible that this period was too short for a complete acclimatisation, which could mean that the temperature change itself influenced the results. Vollrath and co-workers (1997) changed the temperature in one step with 12 °C (from 12 °C to 24 °C), whereas the temperature was only changed with 6 °C (from 14 °C to 20 °C or 20 °C to 26 °C and in reverse direction) in the present study. That the effect of temperature per say is not what is important for web-geometry, but rather the temperature changes experienced previous to web-building, is supported by studies showing that changes in temperature, more than temperature itself, are important in determining the onset of web-building (Witt 1963; Spronk 1935).

Contrary to the web-geometry experiment the web-building behaviour experiment showed clear effects of temperature. There was a decrease in total building time and an increase in speed of construction both during the auxiliary and capture spiral. To my knowledge this is the first study conducted on web-building speed at different temperatures, but Zschokke and Vollrath (1995b) compares speed between two species of orb weavers. They do not state at which temperature their measurements were made, but presumably they were performed at room temperature. For *Araneus diadematus* they find a speed of construction for the auxiliary and capture spiral of 7.6 mm/s and 4.7 mm/s respectively, which agrees reasonably well with the results presented here.

Since metabolic rate is a function of body temperature (Hill and Wyse 1989), it is not surprising that the metabolic rate of spiders show a pronounced dependence on ambient temperature (Anderson 1970). This explains why temperature had such a pronounced effect on web-building speed. However, more than just the actual activity level was affected as is evident from the observed halving in the pause after construction of the auxiliary spiral from 15 °C to 23 °C. This pause is conceivable used by the spider to change from non-sticky to sticky silk production (Gotts and Vollrath 1992). So the lengthening of this pause at lower temperatures indicates that factors such as silk synthesis and silk properties, might also be affected by temperatures within the range encountered by spiders in their natural habitat.

The lack of any effect of temperature on web-geometry found in this study makes it unlikely that adaptation of web-parameters to specific temperatures occur in the wild. The hypothesis that adaptation to larger prey should occur at low temperatures by increasing the mesh size is not supported by the present study. At lower temperatures the spider used longer time to build it webs, and at least in the lab there was no clear indication that the spider compensated for this longer construction time, and thereby greater risk of predation, by building smaller webs. However, more studies with larger sample sizes and greater spans of temperatures are needed before any firm conclusions can be made regarding the validity of these hypotheses. More detailed studies are also needed to investigate the claim made here that the change of temperature might play a bigger role on web-geometry than the actual temperature itself.

References

Anderson, J. F. 1970. Metabolic rates of spiders. *Comparative Biochemistry and Physiology* **33**: 51-72.

Baatrup, E.; Bayley, M. 1993. Quantitative analysis of spider locomotion employing computer-automated video tracking. *Physiology and Behavior* **54**: 83-90.

Barghusen, L. E.; Claussen, D. L. Anderson, M. S.; Bailer, A. J. 1997. The effects of temperature on the web-building behaviour of the common house spider, *Achaearanea tepidariorum*. *Functional Ecology* **11**: 4-10.

Biere, J. M.; Uetz, G. W. 1981. Web orientation in the spider *Micrathena gracilis* (Araneae: Araneidae). *Ecology* **62**: 336-344.

Christiansen, A.; Baum, R.; Witt, P. N. 1962. Changes in webs brought about by mescaline, psilocybin and an increase in body weight. *J. Pharmac. Exp. Ther.* **136**: 31-37.

Eberhard, W. G. 1986. Effects of orb-web geometry on prey interception and retention. From William A. Shear (ed.) *Spiders: webs, behavior and evolution.* P. 70-100.

Stanford University Press. Stanford 1986.

Foelix, R. F. *Biology of spiders 2^nd^ Ed*. Oxford University Press, Oxford 1996.

Fredeen, F. J. H.; Mason, P. G. 1991. Meteorological factors influencing the host-seeking activity of female *Simulium luggeri* (Diptera: Simuliidae). *Journal of Medical Entomology* **28**: 831-840.

Gotts, N. M.; Vollrath, F. 1992. Physical and theoretical features in the simulation of animal behaviour: the spider's web. *Cybernetics and Systems* **23**: 41-65.

Heiling, A. M.; Herberstein, M. E. 1999. The role of experience in web-building spiders (Araneidae). *Animal Cognition* **2**: 171-177.

Herberstein, M. E.; Abernethy, K. E.; Backhouse, K.; Bradford, H.; de Crespigny, F. E.; Luckock, P. R.; Elgar, M. A. 1998. The effect of feeding history on prey capture behaviour in the orb-web spider A*rgiope keyserlingi* Karsch (Araneae: Araneidae). *Ethology* **104**: 565-571.

Herberstein, M. E.; Tso, I-M. 2000. Evaluation of formulae to estimate the capture are and mesh height of orb-webs (Araneoidea, Araneae). *Journal of Arachnology* **28**: 180-184.

Hieber, C. S. 1984. Orb-web orientation and modification by the spiders *Araneus diadematus* and *Araneus gemmoides* (Araneae: Araneidae) in response to wind and light. *Zeitschrift für Tierpsychologie* **65**: 250-260.

Hill, R. W.; Wyse, G. A. 1989. *Animal Physiology*. HarperCollins*Publishers*. New York.

Keselman, H. J. 1998. Testing treatment effects in repeated measures designs: an update for psychophysiological researchers. *Psychophysiology* **35**: 470-478.

Krink, T.; Vollrath, F. 2000. Optimal area use in orb webs of the spider *Araneus diadematus*. *Naturwissenschaften* **87**: 90-93.

Laudien, H. 1973. Activity, behavior etc. From *Temperature and Life* . Eds. Precht, H.; Christophersen, J.; Hensel, H.; Larcher, W. Springer-Verlag Berlin-Heidelberg-New York 1973.

Lehmann, F. O. 1999. Ambient temperature affects free-flight performance in the fruit fly *Drosophila melanogaster*. *Journal of Comparative Physiology B*. **169**: 165-171.

Mayer, G. 1953. Untersuchungen über herstellung und struktur des radnetzes von *Aranea diadema* und *Zilla x-notata* mit besonderer berücksichtigung des unterscheides von jugend- und altersnetzen. *Zeitschrift für Tierpsychologie* **9**: 337-362.

Pedersen, P. O. 1995. Web Extractor: Processing of spider web images. *M.Sc. Thesis*. Department of Computer Science, University of Aarhus, Denmark.

Precht, H.; Christophersen, J.; Hensel, H.; Larcher, W. *Temperature and Life*. Springer-Verlag Berlin-Heidelberg-New York 1973.

Ramousse, R.; Davis, F. 1976. Web-building time in a spider: preliminary applications of ultrasonic detection. *Physiology and Behavior* **17**: 997-1000.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223-225.

Risch, P. 1977. Quantitative analysis of orb web patterns in four species of spiders. *Behavior Genetics* **7**: 199-238.

Sandoval, C. P. 1994. Plasticity in web design in the spider *Parawixia bistriata*: a response to variable prey type. *Functional Ecology* **8**: 701-707.

Schneider, J. M.; Vollrath, F. 1998. The effect of prey type on the geometry of the capture web of *Araneus diadematus*. *Naturwissenschaften* **85**: 391-394.

Sherman, P. M. 1994. The orb-web: an energetic and behavioural estimator of a spider's dynamic foraging and reproductive strategies. *Animal Behaviour* **48**: 19-34.

Spronk, F. 1935. Die abhängigkeit der netsbauzeiten der radnetz-spinnen *Epeira diademata* und *Zilla x-notata* von verschiedenen aussenbedingungen. *Zeitschrift für Vergleichende Physiologie.* **C22**: 604-613.

Uetz, G. W.; Johnson, A. D.; Schemske, D. W. 1978. Web placement, web structure, and prey capture in orb-weaving spiders. *Bulletin of the British Arachnological Society*. **4**: 141-148.

Venner, S.; Pasquet, A.; Leborgne, R. 2000. Web-building behaviour in the orb-weaving spider *Zygiella x-notata*: influence of experience. *Animal Behaviour* **59**: 603-611.

Vollrath, F. 1992. Analysis and interpretation of orb spider exploration and web-building behavior. *Advances in the Study of Behavior* **21**: 147-199.

Vollrath, F.; Downes, M.; Krackow, S. 1997. Design variability in web geometry of an orb-weaving spider. *Physiology and Behavior* **62**: 735-743.

Vollrath, F.; Samu, F. 1997. The effect of starvation on web geometry in an orb-weaving spider. *Bulletin of the British Arachnological Society* **10**: 295-298.

Williams, C. B.; Osman, M. F. H. 1960. A new approach to the problem of the optimum temperature for insect activity. *Journal of Animal Ecology* **29**: 187-189.

Witt, P. N. 1963. Environment in relation to behavior of spiders. *Archives of Environmental Health* **7**: 10-18.

Zschokke, S. 1994. Web construction behaviour of the orb-weaving spider *Araneus diadematus* Cl. *Ph.D. thesis*. Faculty of Philosophy and Science, University of Basel, Switzerland.

Zschokke, S.; Vollrath, F. 1995. Web construction patterns in a range of orb-weaving spiders (Araneae). *European Journal of Entomology* **92**: 523-541.

Zschokke, S.; Vollrath, F. 1995. Unfreezing the behaviour of two orb-web spiders. *Physiology and Behavior* **58**: 1167-1173.

Chapter 4: The effects of neurotoxins on web-geometry and web-building behaviour in *Araneus diadematus* Cl.

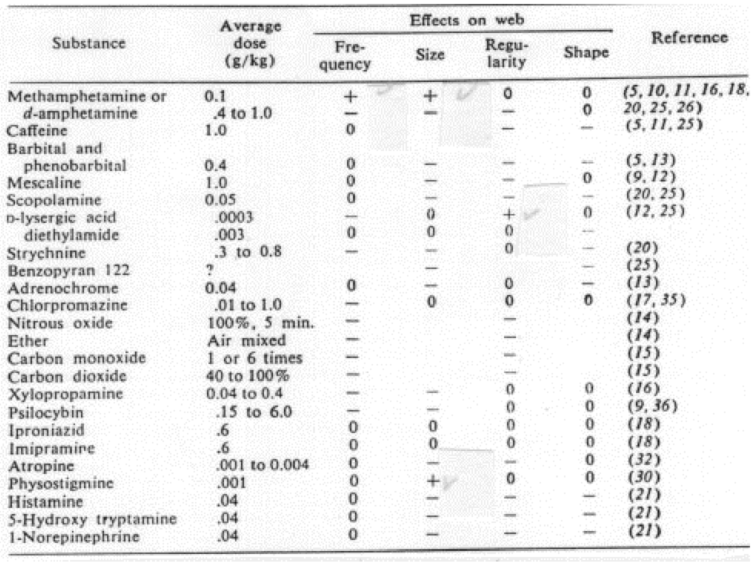
Abstract. The process of orb-weaving and the resultant orb web constitute a good example of a complex behavioural pattern, which is governed by a relatively simple set of rules. The cross spider *Araneus diadematus* was used as a model system to study the effect of three neurotoxins (scopolamine, amphetamine and caffeine) on behaviour decision processes. Scopolamine was given orally at two concentrations, with the lower one showing no effects and the higher resulting in reduced web-building frequency; web-geometry was affected in a very minor way. Amphetamine as well as caffeine resulted in significant changes in both building frequency and web-geometry. Amphetamine webs retained their absolute size but showed a decrease in building efficiency coupled to an increase in spiral spacing (mesh size) and radial irregularity. Caffeine led to smaller but rounder webs coupled to a slight increase in spiral spacing and radial irregularity. Our results indicate that these three general neurotoxins all affect the spider's central nervous system but disturb different parts of the web-building programme.

Introduction

In recent years there has been a growing interest in the field of neuroethology. Most focus has been directed at the invertebrates, whose relatively simple brains and stereotyped behaviour facilitate studies aimed at coupling specific behavioural patterns to distinct neurobiological actions (Simmons and Young 1999). A classical approach has been to administer neuro-active drugs to inhibit certain behaviours and faculties, and thereby learn something about the underlying mechanisms governing the observed behaviour. As our knowledge of both neurobiology and the neurophysiological actions of drugs are increasing, the application of target specific drugs could prove valuable for the further progress of neuroethology.

A promising model organism for neuroethological research is the orb-weaver *Araneus diadematus*. It builds a two-dimensional orb-web, which can be viewed as a fixed record in time of a fairly stereotyped web-building behaviour (Vollrath 1992; Witt et al. 1968; Peters 1939). After a suitable web-site has been located, the actual web-building process can be split up into 4 more or less distinct stages. 1) Creation of the hub and the first radii. 2) Completion of the frame, hub and radii. 3) Construction of the auxiliary spiral. 4) Construction of the capture spiral. During the completion of these stages the spider orientates using a number of sensory cues, such as previously laid threads (Zschokke 1993; Eberhard 1988; Peters 1970), gravity (Vollrath 1988; 1986) and idiothetic memory (Vollrath et al. 2002; Vollrath 1992; Eberhard 1988). Although the web-building behaviour is rather complex, computer simulations have revealed that good approximations of real webs can be accomplished by virtual spiders, which follow only a few simple rules (Krink and Vollrath 1998; 1997; Gotts and Vollrath 1991). The resultant orb-web is a complex geometrical structure, which, however, is easily photographed and analysed for a multitude of components. Furthermore, the geometry of the orb web has proven to be highly sensitive to internal and external factors influencing the spider. These include missing and regenerated legs (Vollrath 1987; Weissmann 1987), age and size (Heiling and Herberstein 1999; Christiansen et al. 1962; Mayer 1953), nutritional state (Herberstein et al. 1998; Vollrath and Samu 1997) ambient temperature and wind (Vollrath et al. 1997) and drug intake (Witt and Reed 1965).

Table 1. The effects of drugs on spiders' web-building behaviour



Substance	Average dose (g/kg)	Effects on web				Reference
		Fre-quency	Size	Regu-larity	Shape	
Methamphetamine or	0.1	+	+	0	0	(5, 10, 11, 16, 18,
d-amphetamine	.4 to 1.0	–	–	–	0	20, 25, 26)
Caffeine	1.0	0		–	–	(5, 11, 25)
Barbital and phenobarbital	0.4	0	–	–	–	(5, 13)
Mescaline	1.0	0	–	–	0	(9, 12)
Scopolamine	0.05	0	–	–	–	(20, 25)
D-lysergic acid	.0003	–	0	+	0	(12, 25)
diethylamide	.003	0	0	0	–	
Strychnine	.3 to 0.8	–	–	0	–	(20)
Benzopyran 122	?		–		–	(25)
Adrenochrome	0.04	0	–	0	–	(13)
Chlorpromazine	.01 to 1.0	–	0	0	0	(17, 35)
Nitrous oxide	100%, 5 min.	–		–		(14)
Ether	Air mixed	–		–		(14)
Carbon monoxide	1 or 6 times	–		–		(15)
Carbon dioxide	40 to 100%	–		–		(15)
Xylopropamine	0.04 to 0.4	–	–	0	0	(16)
Psilocybin	.15 to 6.0	–	–	0	0	(9, 36)
Iproniazid	.6	0	0	0	0	(18)
Imipramine	.6	0	0	0	0	(18)
Atropine	.001 to 0.004	0	–	–	0	(32)
Physostigmine	.001	0	+	0	0	(30)
Histamine	.04	0	–	–	–	(21)
5-Hydroxy tryptamine	.04	0	–	–	–	(21)
1-Norepinephrine	.04	0	–	–	–	(21)

No distinction between spider species was made. Both *Araneus diadematus* and *Zygiella x-notata* were used. Absence of an entry signifies that the particular parameter was not measured. Plus refers to web-building more frequent than controls, larger or more regular webs; minus refers to a decrease in frequency, size or regularity and to a shape different from control; Zero indicates no effect. From Witt and Reed 1965.

Due to the orb-weavers apparent sensitivity to drugs and the ease of detailed web analysis, it was initially hoped that a web-test could be developed (Witt 1971). The idea was to develop a method whereby small quantities, of CNS active substances in body fluids from humans, could be identified by applying the fluids to spiders. From the late 40s until the mid 60s a large range of drugs were administered to spiders and screened for any influence on the orb-webs (see Table 1). Nearly all drugs tested had an effect, and although the web-test proved not to be feasible, these drug-experiments produced valuable insight into the function of a complex behavioural pattern (Witt 1971). At the time the knowledge and techniques available were too limited to allow to relate these findings to specific neurobiological processes. Today, our knowledge of which parts of the central nervous system that are responsible for web-building is still very limited. Only a few such studies have been carried out. One of them, by Weltzien and Barth (1991), failed to find evidence for the hypothesis that the so-called central body, a specialised neuropil in the brain, is important for web-building. However, studies on the wandering spider *Cupiennius salei* have generated important new knowledge on the neurobiology of spiders during the last decades (see Barth 2002 for a review).

As shown in Table 1, a multitude of different drugs have been tested on the spiders and have resulted in more or less profound disruptions in web-geometry. In the present study three of these drugs were chosen for a more detailed study: Scopolamine, methamphetamine (hereafter referred to as amphetamine) and caffeine. These neurotoxins were chosen because of their pronounced, but different effects on the web. The first two drugs were, furthermore, expected to target specific senses in the spider. The anticholinergic alkaloid scopolamine is postulated to disrupt the orientation ability in spiders (Wolff and Hempel 1951) and amphetamine is assumed to disrupt the sensory control (Peters et al. 1950). The last drug, caffeine, is known to affect memory and learning in *Drosophila melanogaster* (Dudai et al. 1986, Folkers and Spatz 1984) and a similar effect might be seen in the spiders. Though, the study was limited to an analysis of web-building behaviour and orb-geometry, a discussion was provided concerning the possible disruption of specific senses by the neurotoxins.

Materials and methods

Experimental design:

Juvenile *Araneus diadematus* spiders were caught at Mols in Eastern Jutland, Denmark. Individual spiders were allowed minimum one week of acclimatisation to standard lab conditions (25° ± 2°C, 45% ± 5% rH, 16/8 L/D), before participating in the experiment. During this period spiders were housed individually in 30 x 30 x 5 cm Perspex frames, separated by thin Vaseline smeared plastic sheets, where they were watered daily and fed one fruit fly per web constructed. Webs were cut each day with a hot wire leaving an intact radius in the north and south quadrant thereby collapsing the web into a single vertical thread, which was left in the frame for the spider to ingest. Only spiders that built three or more consecutive webs were included in the experiment. Five different treatments were used in this experiment with different individual spiders assigned to each treatment. All drug solutions were prepared by dissolving the specific drug in 5.0 mL of distilled water. To facilitate spider ingestion the solutions were sweetened with 1.0 g of fruit sugar (d-Fructose – Sigma-Aldrich Chemie Gmbh, Germany), except for the amphetamine solution where 1.5 g was needed before the spiders accepted the solution. The treatments were: 1) Scopolamine low: 15 mg crystalline scopolamine hydrobromide trihydrate (Fluka Chemika, Switzerland) was dissolved. 2) Scopolamine high: 75 mg crystalline scopolamine hydrobromide trihydrate (Fluka Chemika, Switzerland) was dissolved. 3) Amphetamine: 6 mg crystalline methamphetamine hydrochloride (Sigma-Aldrich Chemie Gmbh, Germany) was dissolved, note that 1.5 g of fruit sugar was added to this solution. 4) Caffeine: 25 mg crystalline caffeine (trimethylxanthine) was dissolved. 5) Control: no drug was added to the solution.

Prior to drug application the webs were placed in the opening of a black back-lit box and photographed with a Nikon Coolpix 995 digital camera. Spiders were then gently removed from the webs and weighed before being returned. When the spiders resettled in the hub, they were fed the drug solution by applying one or two drops, depending on body weight, to the mouth parts using a 5 μL Hamilton Syringe. Drops of various sizes were administered, so that each spider received approximately 0.15 μL solution per mg of body weight. After drug application the spiders were allowed half an hour to ingest the drops before being re-weighed. The spiders were then returned to the webs, which were cut before the frames were placed back under standard lab conditions. The 5 mL solutions consisted of 5 g water and 1 g of sugar plus a small amount of drug, so by applying a density of 1.2 mg per μL, the weight gain of the spiders yields a reliable estimate of the volume of solution ingested. Note that for the amphetamine solution the applied density was 1.3 mg per μL. From this estimate the amount of μg drug intake per mg body weight was calculated. The solutions were always administered in the afternoon and the experimental webs photographed 24 hours later.

The behaviour of the spiders during web-building was recorded for a subset of spiders for the treatments with amphetamine, caffeine and control. The chosen spiders were transferred to the experimental setup, placed in a laboratory with room temperature where artificial light was present to ensure that the dark period was not longer than 8 hours. The setup consisted of two frames placed in the opening of a box with a white background and illuminated from the sides and above by three fluorescent tubes. The tubes emitted only red light, which is invisible for spiders (Baatrup and Bayley 1993), in order to allow recording at night. Two black and white surveillance cameras were fitted in front of the frames and connected to two 'VP112' scanning units (or Baker boxes), which were directly connected to a Macintosh Performa 460 computer. The Baker box scanned the image recorded by the surveillance camera to continuously monitor the position of the spider. Every new position was transferred to the computer running the application Move Recorder for the Macintosh (Zschokke 1994), which stored the positions as x-y co-ordinates. Spiders were allowed several days to acclimate to the experimental setup and were only used in experiments when they had built minimum three webs each. The spiders were administered the drugs using the method described above and returned to the setup. Completed webs were photographed using the method described above.

Data analysis:
The digital images were converted to pixel-graphic and analysed using the software WebExtractor (Pedersen 1995). Additional measurements were obtained with a ruler

from printouts of the photographs. From each web the following data were extracted and analysed.

- Radii length. Total length of all radii.
- Capture length. Total lengths of all capture spiral threads.
- Capture area. The area covered by the capture spiral excluding the hub and free zone.
- No. of reverses per cm spiral thread. During capture spiral-construction the spider reverses its direction of thread laying several times, most often in the southern quadrant where more spiral turns are laid. Since the number of reverses presumably depends on the size of the web, it was here divided by capture length.
- Radial irregularity. The number of Y-shaped (radii that splits into two inside the capture area) and distorted radii as a percentage of total number of radii. The degree of distortion was estimated visually from the printout and the radii was said to be distorted if it deviated with more than around 5 degrees from a straight line.
- Mesh size. Average mesh size was calculated by inserting a vertical line through the hub centre and employing the following formula derived by Herberstein and Tso (2000).

$$\frac{1}{2}\left(\frac{r_u - Hr_u}{S_u - 1} + \frac{r_l - Hr_l}{S_l - 1}\right)$$

Where r_u and r_l are the upper and lower vertical capture spiral lengths. Hr_u and Hr_l are the upper and lower vertical lengths of the hub and the free zone (the area between the hub and the capture spiral) taken together. In this study the centre of the web was defined as the midpoint of the vertical line through the free zone and the hub, thereby keeping Hr_u and Hr_l of equal lengths. S_u and S_l are the numbers of capture spiral turns in the upper and lower part respectively.

- Eccentricity: A measure of the shape of the web, calculated by using the following formula (Vollrath et al. 1997).

$$\sqrt{1 - \left(\frac{L_{hor}}{L_{vert}}\right)^2}$$

Where L_{hor} is the width and L_{vert} the height of the capture spiral. If the width is longer than the height the term in the brackets is inverted and eccentricity assigned a negative value.

From the recordings of movements during web-building the following measurements were obtained and analysed.

- Total building time. The total time elapsed from the spider creating the first radii until finishing the last capture spiral. Pauses during which the spider sits motionless was included in this time.
- Pauses - capture spiral. Number of times the spider was motionless longer than 20s during construction of the capture spiral.
- Speed – auxiliary spiral. The average building speed of the spider when constructing the auxiliary spiral. Pauses were not included in this measurement.
- Speed – capture spiral. The average building speed of the spider when constructing the capture spiral. Pauses were not included in this measurement.
- Pause from aux – cap. The pause between finishing the auxiliary spiral and starting on the first capture spiral turn. This pause is seen in all orb-weavers (Foelix 1996) and could be a necessary break used to change from normal silk to gluey silk.
- Building efficiency. The efficiency exhibited by the spider during capture spiral construction. It was calculated by dividing the length of all spiral threads with the distance the spider had moved during capture spiral construction.

Statistical comparisons between the effects of each drug and the control, on amount of solution ingested and web-building frequency, were made using a two-tailed student t-test for independent samples and a chi-square test with the Yates correction. For all other measurements comparisons were made between the pre-drug webs and the post-drug webs for each individual spider and paired t-tests conducted if possible. In some cases even transformations did not result in equal variances and here the non-parametric Wilcoxon signed rank test for matched samples was used. The radial irregularity was subjected to the arcsine transformation before a paired t-test was attempted. Since several tests were performed on the same data-set, a sequential Bonferroni adjustment was made to minimise type I errors (Rice 1989). The initial significance level was set at 5%. All statistical tests were performed using the software SPSS 10.0 (SPSS Inc. 1999).

Results

Before the chosen parameters of the webs are analysed and compared between pre- and post-drug webs below, it is important to remember that the collected data consisted of photographs. A purely visual based comparison between webs made before and after administration of the control solution revealed, as expected, a high degree of similarity (see Fig. 1A and 1B). However, slight variations in size and especially shape was common, and for 6 out of 23 pairs the difference was large enough to be obvious by visual inspection of the two photographs. For the low and high concentrations of scopolamine, 3 out of 13 and 5 out of 12 respectively showed obvious visual differences. The amphetamine post-drug webs were very characteristically with a large mesh size and slack capture-threads especially towards the periphery (see Fig. 1D). However, 7 out of 22 lacked these differences and were quite similar to the pre-drug webs. For caffeine, the post-drug webs were again characteristically with a clear reduction in size (see Fig. 1F), and here only 3 out of 23 post-drug webs were similar to the pre-drug webs.

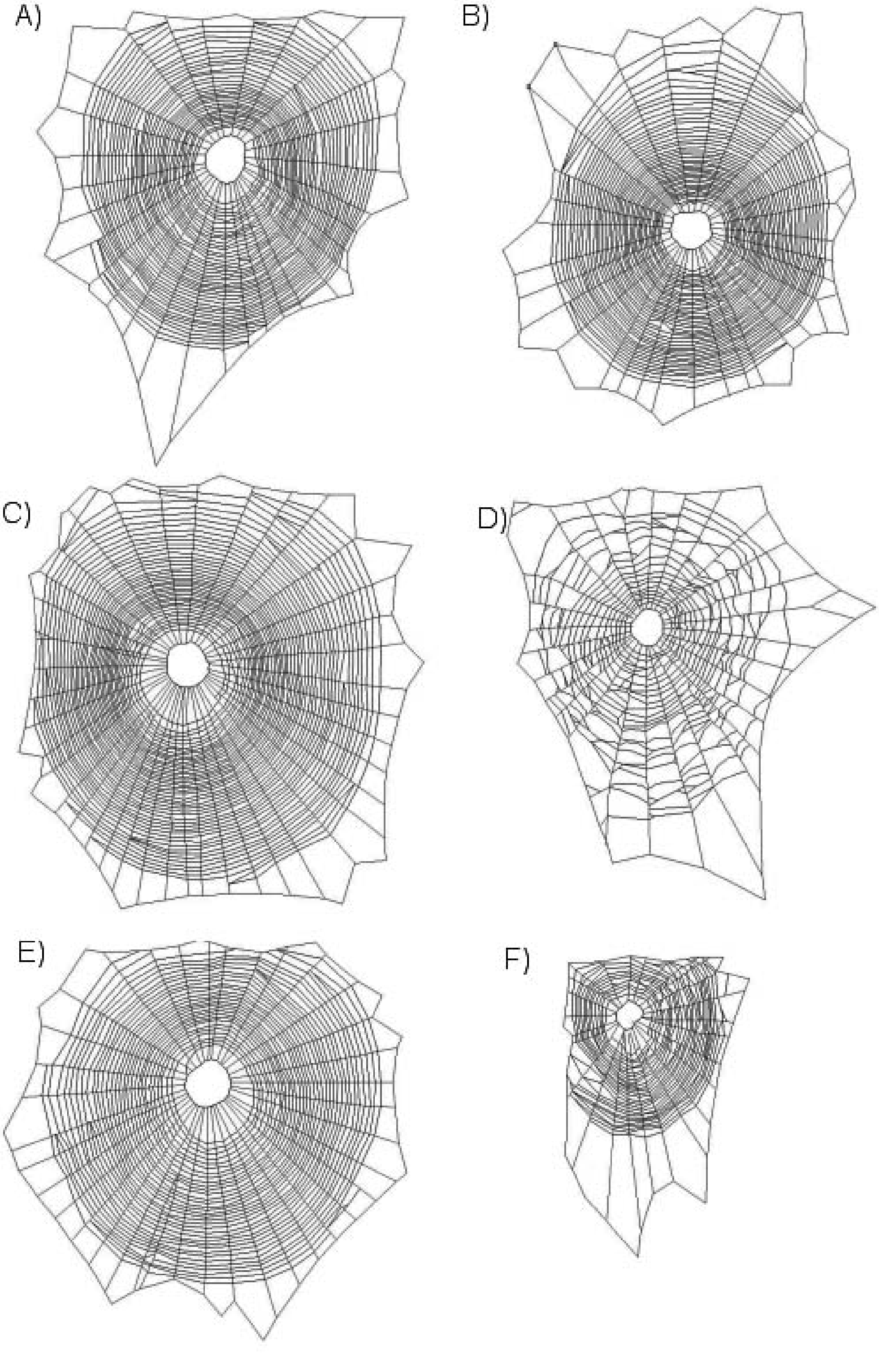


Fig. 1. Digitised photographs of webs from the orb-weaver *Araneus diadematus*. The first column consists of webs made before the spiders were administered a drug solution and the second column are webs from the same spiders made the day after drug application. First row shows webs made before (A) and after (B) administration of a control solution. Second row shows webs made before (C) and after (D) administration of amphetamine. Third row shows webs made before (E) and after (F) administration of caffeine.

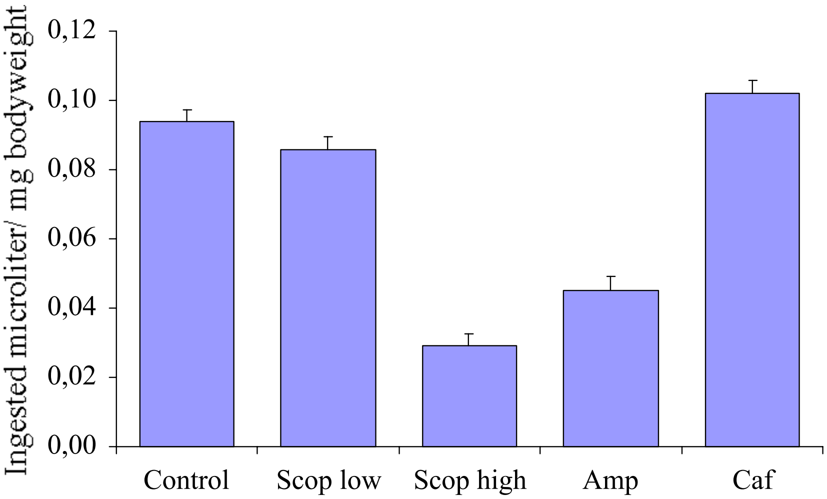


Fig. 2. Amount of solution ingested by the spider *Araneus diadematus*. Control refers to a control solution. Scop low and scop high refer, respectively, to a low and a high concentration of scopolamine. Amp refers to amphetamine and Caf refers to caffeine. Error bars indicate the standard error of the mean.

In Figure 2, the actual amount of drug solution ingested by the spiders for the different treatments is shown. Since approximately 0.15 µL / mg bodyweight drug solution were given to each spider in all treatments, it is evident from Figure 2 that in no cases were all the administered solution ingested. Comparisons of amount ingested between the control and each of the drug solutions showed that administering a high concentration of scopolamine and amphetamine resulted in a significant decrease, whereas a low concentration of scopolamine and caffeine gave no significant difference in amount ingested compared to control (t-test. Low concentration of scopolamine t = 1.54, df = 34, P > 0.050. High concentration of scopolamine t = 12.23, df = 33, P < 0.013. Amphetamine t = 8.96, df = 43, P < 0.017. Caffeine t = -1,61, df = 44, P > 0.025). The results implied that amphetamine and scopolamine in high doses could be detected by the spider and presumably had an adverse taste. Adding more sugar to the solutions could possibly have masked this, but trials with increasing the d-fructose concentration resulted in problems with completely dissolving the sugar and a very viscous solution.

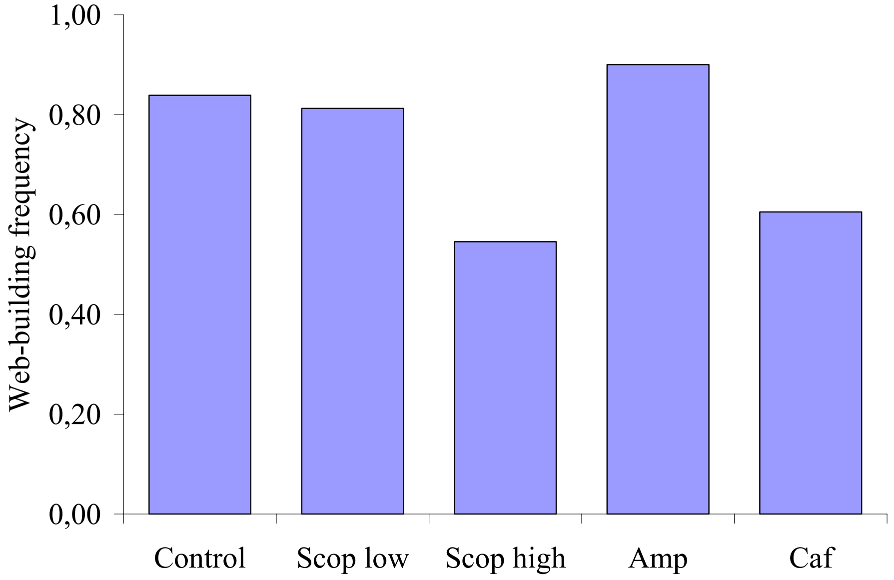


Fig. 3. Web-building frequency of *Araneus diadematus* the day following administration of drugs. For control (Control) 26 out of 31 spiders build a web. For the low concentration of scopolamine (Scop low) 13 out of 16 spiders built a web. For the high concentration of scopolamine (Scop high) 12 out of 22 spiders built a web. For amphetamine (Amp) 27 out of 30 spiders build a web. For caffeine (Caf) 23 out of 38 spiders build a web.

The administered drugs gave rise to different web-building frequencies (see Fig. 3). A statistical analysis revealed that only the high concentration of scopolamine and caffeine differed significantly in web-building frequency compared to the control (χ^2-test. Low concentration of scopolamine $\chi^2 = 0.003$, df = 1, $P > 0.050$. High concentration of scopolamine $\chi^2 = 11.9$, df = 1, $P < 0.017$. Amphetamine $\chi^2 = 0.44$, df = 1, $P > 0.025$. Caffeine $\chi^2 = 13.6$, df = 1, $P < 0.013$). Although not significant, the results indicate that amphetamine actually had a higher web-building frequency than the control.

The effects of the drugs on the web-geometry are summarised in Tables 3-6. Table 2 shows that there were no significant effects on the webs by application of the control solution. Administration of a low concentration of scopolamine did not result in any significant effects on any of the web-parameters analysed (see Table 3), although a slight increase in radial irregularity was detectable. The high concentration of scopolamine had no significant effects either (see Table 4), however, it doubled the radial irregularity compared with the pre-drug webs and resulted in a decrease in radii- and capture spiral length and a reduction of capture spiral area. Amphetamine had severe effects on web-geometry (see Table 5), resulting in a significant threefold rise in radial irregularity. The length of all capture spiral threads was significantly reduced, whereas the area of the capture spiral was unchanged. This led to a significantly larger mesh size in the spiral. Application of caffeine also resulted in profound changes in web-geometry (see table 6). Total length of all radii, the length of the capture spiral and the area of the capture spiral were all significantly reduced. The radial irregularity was almost twice as high compared to pre-drug webs. The eccentricity of the webs was significantly lower, resulting in more round webs. Although there was no significant change in the number of reverses, the data did indicate a rise. Mesh size was also slightly larger in the post-drug webs, although this was not statistical significant.

In Tables 8 and 9, the effects of amphetamine and caffeine on the behaviour of the web-building spider are summarised. As scopolamine showed no significant effects on web-geometry, it was decided not to record its effects on web-building behaviour. Table 7 shows that there were no effects of the control solution on web-building behaviour. The relatively large decrease in total web-building time observed in the post-treatment webs, was mostly the result of fewer pauses during radial and frame construction, which is also the stage in the web-building sequence known to exhibit highest variation (Vollrath 1992). A larger sample size would presumably remove this effect. Note the unrealistically high capture spiral building efficiency observed throughout this study. This could result from either an overestimation of capture spiral length made by the digitising software (WebExtractor) or the fact that not all movements made during the construction of the capture spiral were actually recorded by the Baker boxes. The application of both amphetamine and caffeine showed visual effects on the spiders within an hour resulting in more sluggish and passive spiders. This immediate effect, however, did not result in delay in onset of web-building, as the time from application of the drug-solution to the onset of web-building was roughly the same for the drugged and control spiders (see Tables 7,8 and 9).

For amphetamine, the significant effects on web-building behaviour were a reduction in speed both during auxiliary and capture spiral construction (see Table 8).

There was also a clear reduction in capture spiral building efficiency, although this was exactly not statistical significant. The data also indicated an increase in total building time. Caffeine resulted in a reduction of speed during both auxiliary and spiral construction (see Table 9). However, contrary to amphetamine, caffeine led to a significant increase in the pause after finishing the auxiliary spiral and before commencing on the capture spiral. Caffeine, furthermore, did not result in changes in total building time or capture spiral building efficiency, although the latter was close to being statistically significant.

Table 2. The effects of administration of a control solution on web-geometry

	Pre-drug treatment	Post-drug treatment	Test	P		Adjusted α*
Number of spiders	23					
Weight in mg	35.45 ± 2.17	39.56 ± 2.50				
Radii length in mm	2837 ± 143	2787 ± 198	$t_{22} = 0.31$[a]	0.756	>	0.017
Capture spiral length in mm	10826 ± 715	10334 ± 801	$t_{22} =0.73$[a]	0.474	>	0.010
Capture area in mm^2	23933 ± 1570	22748 ± 1716	$t_{22} = 0.81$[a]	0.425	>	0.008
Reverses per m spiral thread	0.64 ± 0.07	0.68 ± 0.09	$t_{22} = -0.43$[a]	0.669	>	0.013
Radial irregularity in %	25.17 ± 1.81	25.26 ± 2.37	$t_{22} = -0.03$[a]	0.976	>	0.050
Mesh size in mm	2.33 ± 0.07	2.34 ± 0.10	$t_{22} = -0.15$[a]	0.881	>	0.025
Eccentricity	0.47 ± 0.04	0.39 ± 0.05	$t_{22} = 1.69$[a]	0.106	>	0.007

All values are given with the standard error of the mean.
* Since several tests were performed on the same data-set, the significance levels were adjusted using the sequential Bonferroni method.
a. The two-tailed paired t-test.

Table 3. The effects of administration of a low concentration of scopolamine on web-geometry

	Pre-drug treatment	Post-drug treatment	Test	P		Adjusted α*
Number of spiders	13					
Weight in mg	28.53 ± 1.31	31.48 ± 1.48				
Drug intake in μg/mg	-	0.26 ± 0.01				
Radii length in mm	2593 ± 251	2448 ± 198	$t_{12} = 1.12$[a]	0.287	>	0.010
Capture spiral length in mm	9318 ± 897	8456 ± 788	$t_{12} = 1.57$[a]	0.143	>	0.007
Capture area in mm^2	31012 ± 3106	30283 ± 2943	$t_{12} = 0.44$[a]	0.667	>	0.025
Reverses per m spiral thread	0.68 ± 0.11	0.65 ± 0.09	$t_{12} = 0.38$[a]	0.709	>	0.050
Radial irregularity in %	7.95 ± 1.54	10.92 ± 3.54	$t_{12} = -0.68$[a]	0.507	>	0.017
Mesh size in mm	2.36 ± 0.12	2.54 ± 0.13	$t_{12} = -1.48$[a]	0.164	>	0.008
Eccentricity	0.57 ± 0.02	0.50 ± 0.07	$t_{12} = 1.07$[a]	0.307	>	0.013

All values are given with the standard error of the mean.
* Since several tests were performed on the same data-set, the significance levels were adjusted using the sequential Bonferroni method.
a. The two-tailed paired t-test.

Table 4. The effects of administration of a high concentration of scopolamine on web-geometry

	Pre-drug treatment	Post-drug treatment	Test	P		Adjusted α^*
Number of spiders	12					
Weight in mg	36.45 ± 2.26	37.75 ± 2.39				
Drug intake in µg/mg	-	0.44 ± 0.05				
Radii length in mm	2471 ± 208	2067 ± 165	$t_{11} = 1.71^a$	0.114	>	0.010
Capture spiral length in mm	10145 ± 992	8007 ± 683	$t_{11} = 2.30^a$	0.042	>	0.007
Capture area in mm^2	31512 ± 2749	26410 ± 2475	$t_{11} = 1.62^a$	0.134	>	0.013
Reverses per m spiral thread	0,72 ± 0.12	0.75 ± 0.08	$t_{11} = -0.19^a$	0.851	>	0.050
Radial irregularity in %	12.29 ± 1.64	24.49 ± 5.94	$t_{11} = -2.19^a$	0.051	>	0.008
Mesh size in mm	2.41 ± 0.16	2.39 ± 0.16	$t_{11} = 0.21^a$	0.838	>	0.025
Eccentricity	0.57 ± 0.06	0.61 ± 0.02	$Z = -0.51^b$	0.610	>	0.017

All values are given with the standard error of the mean.

* Since several tests were performed on the same data-set, the significance levels were adjusted using the sequential Bonferroni method.

a. The two-tailed paired t-test.

b. The Wilcoxon's signed rank test.

Table 5. The effects of administration of amphetamine on web-geometry

	Pre-drug treatment	Post-drug treatment	Test	P		Adjusted α^*
Number of spiders	22					
Weight in mg	33. 07 ± 1.78	34.99 ± 1.89				
Drug intake in µg/mg	-	0.056 ± 0.005				
Radii length in mm	3020 ± 163	2970 ± 129	$t_{21} = 0.25^a$	0.809	>	0.05
Capture spiral length in mm	11735 ± 695	7515 ± 590	$t_{21} = 4.53^a$	$1x10^{-4}$	<	0.008
Capture area in mm^2	25408 ± 1739	24118 ± 1399	$t_{21} = 0.66^a$	0.514	>	0.025
Reverses per m spiral thread	0.63 ± 0.08	0.50 ± 0.07	$t_{21} = 1.45^a$	0.161	>	0.013
Radial irregularity in %	20.00 ± 1.81	64.36 ± 6.23	$Z = -3.69^b$	$2x10^{-4}$	<	0.010
Mesh size in mm	2.24 ± 0.07	4.26 ± 0.46	$Z = -4.11^b$	$4x10^{-5}$	<	0.007
Eccentricity	0.50 ± 0.04	0.44 ± 0.08	$Z = -0.66^b$	0.509	>	0.017

All values are given with the standard error of the mean.

* Since several tests were performed on the same data-set, the significance levels were adjusted using the sequential Bonferroni method.

a. The two-tailed paired t-test.

b. The Wilcoxon's signed rank test

Table 6. The effects of caffeine on web-geometry

	Pre-drug treatment	Post-drug treatment	Test	P		Adjusted α^*
Number of spiders	23					
Weight in mg	37.01 ± 7.88	41.59 ± 9.11				
Drug intake in µg/mg	-	0.256 ± 0.009				
Radii length in mm	2959 ± 212	1877 ± 170	$t_{22} = 6.67^a$	$1x10^{-6}$	<	0.010
Capture spiral length in mm	12122 ± 1100	5373 ± 791	$t_{22} = 7.98^a$	$6x10^{-8}$	<	0.007
Capture area in mm^2	24652 ± 2018	12102 ± 1395	$t_{22} = 7.76^a$	$1x10^{-7}$	<	0.008
Reverses per m spiral thread	0.67 ± 0.08	0.84 ± 0.09	$t_{22} = -1.68^a$	0.108	>	0.050
Radial irregularity in %	23.61 ± 1.94	40.04 ± 2.86	$t_{22} = -5.57^a$	$1x10^{-5}$	<	0.013
Mesh size in mm	2.19 ± 0.08	2.72 ± 0.20	$Z = -2.24^b$	0.025	>	0.025
Eccentricity	0.49 ± 0.04	0.21 ± 0.09	$Z = -2.69^b$	0.007	<	0.017

All values are given with the standard error of the mean.
* Since several tests were performed on the same data-set, the significance levels were adjusted using the sequential Bonferroni method.
a. The two-tailed paired t-test
b. The Wilcoxon's signed rank test

Table 7. The effect of administration of a control solution on web-building behaviour

	Pre-drug treatment	Post-drug treatment	Test	P		Adjusted α^*
Number of spiders	6					
Delay time in hours$^\#$	-	18.5 ± 1.8				
Total building time in min.	53.0 ± 6.0	40.5 ± 5.2	$t_5 = 1.53^a$	0.186	>	0.017
Number of pauses - capture	0	0				
Speed – auxiliary in mm/s	7.1 ± 0.3	8.3 ± 0.7	$t_5 = -2.00^a$	0.101	>	0.010
Speed capture in mm/s	5.4 ± 0.3	5.7 ± 0.5	$t_5 = -0.95^a$	0.385	>	0.025
Pause from aux to cap in sec.	28 ± 4	26 ± 3	$t_5 = 0.37^a$	0.725	>	0.050
Building efficiency in %	95.33 ± 1.5	99.00 ± 1.46	$t_5 = -1.64^a$	0.161	>	0.013

All values are given with the standard error of the mean.
Delay time is the duration from drug administration until onset of web-building.
* Since several tests were performed on the same data-set, the significance levels were adjusted using the sequential Bonferroni method.
a. The two-tailed paired t-test.

Table 8. The effect of administration of amphetamine on web-building behaviour

	Pre-drug treatment	Post-drug treatment	Test	P		Adjusted α[*]
Number of spiders	8					
Drug intake in µg/mg	-	0.061 ± 0.006				
Delay time in hours[#]	-	16.7 ± 0.8				
Total building time in min.	51.5 ± 9.0	76.1 ± 11.6	$t_7 = -2.44$[a]	0.045	>	0.025
Number of pauses - capture	0	2.3 ± 1.4				
Speed – auxiliary in mm/s	11.0 ± 0.6	9.0 ± 0.4	$t_7 = 1.93$[a]	0.005	<	0.013
Speed capture in mm/s	6.6 ± 0.2	4.9 ± 0.3	$t_7 = 4.66$[a]	0.002	<	0.010
Pause from aux to cap in sec.	18 ± 2	31 ± 10	$Z = -1.47$[b]	0.141	>	0.050
Building efficiency in %	101.0 ± 1.5	71.8 ± 10.5	$Z = -2.38$[b]	0.017	>	0.017

All values are given with the standard error of the mean.

Delay time is the duration from drug administration until onset of web-building.

* Since several tests were performed on the same data-set, the significance levels were adjusted using the sequential Bonferroni method.

a. The two-tailed paired t-test.

b. The Wilcoxon's signed rank test

Table 9. The effect of administration of caffeine on web-building behaviour

	Pre-drug treatment	Post-drug treatment	Test	P		Adjusted α[*]
Number of spiders	7					
Drug intake in µg/mg	-	0.266 ± 0.014				
Delay time in hours[#]	-	16.5 ± 0.8				
Total building time in min.	48.3 ± 6.8	46.5 ± 6.0	$t_6 = 0.19$[a]	0.859	>	0.050
Number of pauses - capture	0	0				
Speed – auxiliary in mm/s	8.8 ± 0.6	5.4 ± 1.0	$t_6 = 3.70$[a]	0.010	<	0.013
Speed capture in mm/s	6.4 ± 0.4	4.5 ± 0.6	$t_6 = 4.10$[a]	0.006	<	0.010
Pause from aux to cap in sec.	20 ± 1	64 ± 34	$Z = -2.20$[b]	0.028	>	0.017
Building efficiency in %	103.7 ± 1.9	92.9 ± 5.7	$Z = -1.99$[b]	0.046	>	0.025

All values are given with the standard error of the mean.

Delay time is the duration from drug administration until onset of web-building.

* Since several tests were performed on the same data-set, the significance levels were adjusted using the sequential Bonferroni method.

a. The two-tailed paired t-test.

b. The Wilcoxon's signed rank test

Discussion

Orb-weavers exhibit a high degree of individual variation in their responses to biotic and abiotic factors (Heiling and Herberstein 2000; Sherman 1994). It is therefore not surprising that *Araneus diadematus* showed individual variation in the effects of the neurotoxins examined here. Some individuals even built seemingly normal webs after ingestion of the same doses at which other individuals built highly distorted webs. Despite of this the results of the present study agree with earlier work on spiders' responses to drugs (see Witt and Reed 1965 for a review) in that drug-specific actions were found on the geometry of the orb-web. The experiments on scopolamine conducted in this study showed that web-building frequency was dose dependent and indicated that the higher concentration, and higher drug intake, influenced several web-parameters, whereas the lower concentration of scopolamine showed no such influence. Dose dependent effects have been reported for a wide range of drugs (Reed and Witt 1968; Christiansen et al. 1962; Wolff and Hempel 1951). The concentrations chosen in this study were a compromise between the wish to observe actual changes on one hand and on the other hand to ensure that the drug solution was readily ingested by the spider and that web-building frequency was high enough to ensure an appropriate sample size. Samu and Vollrath (1992) used a different approach to ensure ready acceptance of liquid pesticides sweetened with sugar. They deprived the spiders of water for 3-5 days prior to drug application. This approach was discarded in this study as it was feared that water deprivation would result in an increased variation in web-parameters and that the sudden ingestion, of much solution on the day of drug application, could lead to differences between pre- and post administration webs in the control experiment.

Administration of a low concentration of scopolamine resulted in an actual intake by the spiders of around 0.26 µg scopolamine per mg spider body weight. This dose had no effect on either web-building frequency or any of the web-parameters investigated. Raising the concentration of scopolamine fivefold resulted only in a rise of intake to 0.44 µg/mg due to a much lower amount of drug solution actually ingested by the spiders. However, this rise in dose was enough to significantly reduce web-building frequency and, although not statistical significant, there was an indication that it also reduced web-size and led to a higher radial irregularity. It would have been interesting to apply higher concentrations of scopolamine. However, trial experiment resulted in active rejection of the drops attached to the mouth parts. The spiders moved out of the hub and wiped off the drops. Earlier work on the effect of scopolamine was conducted on adult *Zygiella x-notata*. Wolff and Hempel (1951) do not state the actual dose given per mg bodyweight, but a calculation from their average dose applied and the mean weight of adult female *Zygiella x-notata* gave a dose of around 0.7 µg/mg. Witt and Reed (1965) mention a dose as low as 0.05 µg /mg, however, as their paper is a review (one of the papers reviewed was Wolff and Hempel's study mentioned above), this figure is questionably. After administration of scopolamine they found no change in web-building frequency, an increase in eccentricity and a decrease in both size and regularity. The first two results are in disagreement with the findings of the present study, where there was a clear reduction in web-building frequency and no sign of an increase in eccentricity. However, this might be explained by the use of juvenile *Araneus diadematus* instead of adult *Zygiella x-notata*.

In humans, scopolamine is known to antagonise acetylcholine at the muscarinic receptors in the CNS (Kopelman 1986) and has negative effects on memory and learning in monkeys and humans (Harder et al. 1998, Kopelman 1986). Similar effects are also reported in octopuses (Fiorito et al. 1998). In spiders acetylcholine is found in the CNS (Fabian and Seyfarth 1997; Meyer et al 1984) and the enzyme acetylcholinesterase, which destroys acetylcholine, has been found in high concentrations in parts of the brain thought to be associated with motor control for orb-weaving (Meyer and Pospiech 1977). Scopolamine is therefore expected to have a significant impact on spider behaviour and result in changes in orb-web geometry. The relative weak effects observed in this study might be due to a too low dose given. However, it is also possible that the method of oral application is not well suited for this particular drug. Larvae of the pest *Spodoptera littoralis* show no adverse effect when eating plant material containing high concentrations of scopolamine (Krug and Proksch 1993). The authors speculate that this could be due to insufficient permeability of scopolamine through the gut membrane.

The concentration of amphetamine given in this experiment led to a drug intake of 0.06 μg/mg and resulted in a decrease in capture spiral length, but no change in radii length or capture spiral area. Furthermore, an increase in radial irregularity and mesh size was observed. An analysis of the movements made by the spider during web-building showed a clear reduction in speed both during auxiliary and capture spiral construction compared to the control. Interestingly enough, there also seemed to be a reduction in capture spiral construction efficiency, albeit not significantly so, where the spider moved longer, compared to the control, for laying down the same length of spiral thread. Older studies on adult *Araneus diadematus* and *Zygiella x-notata* showed that a low dose of 0.1 μg/mg resulted in an increase in web-building frequency and web size, whereas a high dose of 0.4 – 1.0 μg/mg resulted in a decrease in web-building frequency, in the number of radii and spiral turns, in capture area and in regularity (Reed et al. 1982; Witt and Reed 1965). This is in partial agreement with the results presented in this study, although no decrease in capture area or radii length was found here.

Amphetamine is reported to disrupt the sensory control of the spider and causing it sometimes to reach into empty air when using the previously laid capture spiral for construction of the next (Peters et al. 1950). Mesh size is fairly constant in the orb web, however, the outer spirals often show an increase in mesh size (ap Rhisiart and Vollrath 1994, Witt 1952). As previously laid capture spiral turns are used during construction (Vollrath 1986), it is expected that webs build by spiders experiencing sensory problems would show the largest disruption towards the periphery. This is indeed what was observed in this study for webs made after administration of amphetamine. An alternative explanation is that the drug was causing a shortening of the auxiliary spiral and thereby resulting in problems for the spider to lay down the spiral turns at the periphery. However, no such shortening was evident in this study in a comparison between pre- and post-amphetamine webs. The increase in radial irregularity and especially the decrease in capture spiral construction efficiency found in this study further indicates that amphetamine drugged spiders were experiencing problems with integrating sensory inputs.

In humans, amphetamine primarily destroys the dopaminergic and serotonergic terminals in the CNS, thereby causing release of the nerurotransmitters dopamine and serotonin (Davidson et al. 2001; Frost and Cadet 2000). Dopamine and serotonin are both found in the spider CNS (Schmid et al. 1992). Serotonin seems to exert many different actions, but its main function is probably to transfer information from the afferent of the mechano- and chemoreceptive sensory hairs (Barth 2002). To our knowledge there is as yet no available information on the role of dopamine in the CNS of spiders, however, it is known to increase motivation in mammals (Bradford 1986). A similar effect in spiders may be responsibly for the indicated increase in web-building frequency after administration of amphetamine found in this study and reported by Witt and Reed (1965).

The caffeine solution used here resulted in an average dose of 0.26 μg/mg. This caused a decrease in radii length, capture spiral length and capture spiral area. There was also a slight increase in radial irregularity and mesh size, although not as much as that observed for amphetamine. Webs made by caffeine drugged spider were characteristically more round than control webs. Furthermore, it caused a reduction in speed during both auxiliary and capture spiral construction. It is noteworthy that caffeine seemed to prolong the pause between auxiliary and spiral construction. Caffeine is claimed to be one of the most potent of the drugs tested on orb-weavers (Witt et al. 1968; Witt and Read 1965; Peters et al. 1950). They found that a dose of 1.0 μg/mg caused a decrease in capture spiral area, led to more round webs and higher radial irregularity. Furthermore, they observed an increase in web-building time. These observations are in good agreement with the findings in the present study, although they found more profound effects of caffeine on web-geometry. This may be due to their use of a more than three-fold higher dose. However, unpublished trial experiments applying a dose of 0.42 μg caffeine per mg spider weight resulted in a web-building frequency of virtually zero and the death of half of the drugged spiders within a week.

Caffeine antagonises adenosine receptors in the human CNS (Sawynok and Yaksh 1993). To our knowledge there are no published information about the role of adenosine in the spider CNS, but adenosine diphosphate (ADP) and adenosine triphosphate (ATP) play a fundamental role in the energetics of all living organisms. However, caffeine exerts different actions in man and spider. Caffeine has the effect, among others, on humans that it improves motor performance (Sawynok and Yaksh 1993), whereas it on spiders, as the present study shows, acts exactly opposite by reducing the web-building speed.

The experiments described in this study lent support to the hypothesis that neurotoxins affect the web-geometry and web-building behaviour of *Araneus diadematus* in a drug specific way. The results were inconclusive concerning any significant effects of scopolamine, but clearly supported the hypothesis that amphetamine and caffeine have profound and distinct effects. However, the present study did not allow for conclusions about which exact senses or faculties were targeted by the drugs. The hypothesis that scopolamine affects the sense of orientation could not be verified at the concentrations used here. The data provided some support to the notion that amphetamine disrupts motor sensory control. Caffeine disrupted the web-building programme, but whether it caused this by affecting the memory of the spider is presently unknown. Amphetamine seemed to be the drug with the greatest potential. However, even such a powerful drug, which caused profound changes in the capture spiral, did not cause any significant changes in the auxiliary spiral. Recording of the movements made during auxiliary spiral constructions revealed, besides a reduction of construction speed, no visible changes to the path of the auxiliary spiral. It appeared to be just as regular after intake of amphetamine as before. Since the auxiliary spiral is used as a handrail during construction of the capture spiral (Zschokke and Vollrath 1995; Zschokke 1993), it would be interesting to find a drug that cause a disruption of the auxiliary spiral. Scopolamine is described as having such effects (Wollf and Hempel 1951), however, the results of the present study did not support this observation.

Further experiments on other parts of spider behaviour, like predatory behaviour, escape movements etc, could provide valuable additional information about which senses might be affected by the neurotoxins. Another interesting approach would be to use radioactive labelled drugs, which would allow determination of where in the spider CNS the drugs are concentrated. Together with neurophysiological methods this could yield insight into which parts of the brain are important for which steps in the web-building process. The application of drugs to orb-weavers described in this study could, with the right integrative approach, prove to be a valuable new model system to generate new insights into invertebrate neuroethology.

Reference

Ap Rhisiart, A.; Vollrath, F. 1994. Design features of the orb web of the spider, *Araneus diadematus*. *Behavioral Ecology* **5**: 280-287.

Baatrup, E.; Bayley, M. 1993. Quantitative analysis of spider locomotion employing computer-automated video tracking. *Physiology and Behavior* **54**: 83-90.

Barth, F. *A spider's world: senses and behaviour*. Springer-Verlag. Berlin. 2002.

Bradford, H. F. *Chemical neurobiology: an introduction to neurochemistry*. W. H. Freeman and Company. New York. 1986.

Christiansen, A.; Baum, R.; Witt, P. N. 1962. Changes in spider webs brought about by mescaline, psilocybin and an increase in body weight. *J. Pharmac. Exp. Ther.* **136**: 31-37.

Davidson, C.; Gow, A. J.; Lee, T. H.; Ellinwood, E. H. 2001. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. *Brain Research Reviews* **36:** 1-22.

Dudai, Y.; Buxbaum, J.; Corfas, G.; Orgad, S.; Segal, D.; Sher, B.; Uzzan, A.; Zvi, S. 1986. Defective cAMP metabolism and defective memory in Drosophila. *Acta Biochem. Biophys. Hung.* **21**: 177-192.

Eberhard, W. G. 1988. Memory of distances and directions moved as cues during temporary spiral construction in the spider *Leucage mariana* (Araneae: Araneidae). *Journal of Insect Behavior* **1**: 51-66.

Fabian, R.; Seyfarth, E-A. 1997. Acetylcholine and histamine are transmitter candidates in identifiable mechanosensitive neurons of the spider *Cupiennius salei*: an immunocytochemical study. *Cell & Tissue Research* **287**: 413-423.

Fiorito, G.; Agnisola, C.; d'Addio, M.; Valanzano, A.; Calamandrei, G. 1998. Scopolamine impairs memory recall in *Octopus vulgaris*. *Neuroscience Letters* **253:** 87-90.

Foelix, R. F. *Biology of spiders 2nd Ed*. Oxford University Press, Oxford 1996.

Folkers, E.; Spatz, H. C. 1984. Visual learning performance of *Drosophila melanogaster* is altered by neuropharmaca affecting phosphodiesterase and acetylcholine transmission. *Journal of Insect Physiology* **30**: 957-965.

Frost, D. O.; Cadet, J-L. 2000. Effects of methamphetamine-induced neurotoxicity on the development of neural circuitry: a hypothesis. *Brain Research Reviews* **34:** 103-118.

Gotts, N. M.; Vollrath, F. 1991. Artificial intelligence modelling of web-building in the garden cross spider. *Journal of Theoretical Biology* **152**: 485-511

Harder, J. A.; Baker, H. F.; Ridley, R. M. 1998. The role of the central cholinergic projections in cognition: implications of the effects of scopolamine on discrimination learning by monkeys. *Brain Research Bulletin* **45:** 319-326.

Heiling, A. M.; Herberstein, M. E. 1999. The role of experience in web-building spiders (Araneidae). *Animal Cognition* **2**: 171-177.

Heiling, A. M.; Herberstein, M. E. 2000. Interpretations of orb-web variability: A review of past and current ideas. *Ekología* **19:** 97-106.

Herberstein, M. E.; Abernethy, K. E.; Backhouse, K.; Bradford, H.; de Crespigny, F. E.; Luckock, P. R.; Elgar, M. A. 1998. The effect of feeding history on prey capture behaviour in the orb-web spider A*rgiope keyserlingi* Karsch (Araneae: Araneidae). *Ethology* **104**: 565-571.

Herberstein, M. E.; Tso, I-M. 2000. Evaluation of formulae to estimate the capture area and mesh height of orb-webs (Araneoidea, Araneae). *Journal of Arachnology* **28**: 180-184.

Kopelman, M. D. 1986. The cholinergic neurotransmitter system in human memory and dementia: a review. *The Quarterly Journal of Experimental Psychology* **38A:** 535-573.

Krink, T.; Vollrath, F. 1997. Analysing spider web-building behaviour with rule-based simulations and genetic algorithms. *Journal of Theoretical Biology* **185**: 321-331

Krink, T.; Vollrath, F. 1998. Emergent properties in the behaviour of a virtual spider robot. *Proceedings of the Royal Society. London. Series B* **265**: 2051-2055.

Krug, E.; Proksch, P. 1993. Influence of dietary alkaloids on survival and growth of *Spodoptera littoralis*. *Biochemical Systematics and Ecology* **21:** 749-756.

Mayer, G. 1953. Untersuchungen über herstellung und struktur des radnetzes von *Aranea diadema* und *Zilla x-notata* mit besonderer berücksichtigung des unterscheides von jugend- und altersnetzen. *Zeitschrift für Tierpsychologie* **9**: 337-362.

Meyer, W.; Pospiech, B. 1977. The distribution of acetylcholinesterase in the central nervous system of web-building spiders (*Arachnida, Araneae*). *Histochemistry* **51:** 201-208.

Meyer, W.; Schlesinger, C.; Poehling, H. M.; Ruge, W. 1984. Comparative quantitative aspects of putative neurotransmitters in the central nervous system of spiders (Arachnida: Araneidae). *Comparative Biochemistry and Physiology*. **78C:** 357-362.

Pedersen, P. O. 1995. Web Extractor: Processing of spider web images. *M.Sc. Thesis*. Department of Computer Science, University of Aarhus, Denmark.

Peters, H. M. 1939. Über das kreuzspinnennetz und seine probleme. *Naturwissenschaften* **47:** 777-786

Peters, H. M.; Witt, P. N.; Wolff, D. 1950. Die beeinflussung des netzbaues der spinnen durch neurotrope substanzen. *Zeitschrift für Vergleichende Physiologie* **32**: 29-45.

Peters, P. J. 1970. Orb-web construction: interaction of spider (*Araneus diadematus* Cl.) and thread configuration. *Animal Behaviour* **18**: 478-484.

Reed, C. F.; Witt, P. N. 1968. Progressive disturbance of spider web geometry caused by two sedative drugs. *Physiology and Behavior* **3:** 119-124.

Reed, C. F.; Witt, P.N.; Scarboro, M. B. 1982. Maturation and *d*-amphetamine – induced changes in web building. *Developmental Psychobiology* **15**: 61-70.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223-225.

Samu, F.; Vollrath, F. 1992. Spider orb web as bioassay for pesticide side effects. *Entomol. Exp. Appl.* **62**: 117-124.

Sawynok, J.; Yaksh, T. L. 1993. Caffeine as an analgesic adjuvant: a review of pharmacology and mechanisms of action. *Pharmacological Reviews* **45**: 43-85.

Schmid, A.; Sperk, G.; Reither, H. 1992. Quantitative determination of neuroactive substances in the CNS of the spider *Cupiennius salei* KEYS. *Comparative Biochemistry and Physiology* **102C:** 447-450.

Sherman, P. M. 1994. The orb-web: an energetic and behavioural estimator of a spider's dynamic foraging and reproductive strategies. *Animal Behaviour* **48**: 19-34.

Simmons, P.; Young, D. *Nerve cells and animal behaviour. 2^{nd} edition*. Cambridge University Press. UK. 1999.

Vollrath, F. 1986. Gravity as an orientation guide during web-construction in the orb spider *Araneus diadematus* (Araneae, Araneidae). *Journal of Comparative Physiology A* **159**: 275-280.

Vollrath, F. 1987. Altered geometry of webs in spiders with regenerated legs. *Nature* **328**: 247-248.

Vollrath, F. 1988. Spiral orientation of *Araneus diadematus* orb webs built during vertical rotation. *Journal of Comparative Physiology A* **162**: 413-419.

Vollrath, F. 1992. Analysis and interpretation of orb spider exploration and web-building behavior. *Advances in the Study of Behavior* **21**: 147-199

Vollrath, F.; Downes, M.; Krackow, S. 1997. Design variability in web geometry of an orb-weaving spider. *Physiology and Behavior* **62**: 735-743.

Vollrath, F.; Nørgaard, T.; Krieger, M. Radius orientation in the cross spider *Araneus diadematus*. From *European Arachnology 2000* (S. Toft and N. Scharff eds.) pp 107-116. Aarhus University Press, Aarhus, 2002.

Vollrath, F.; Samu, F. 1997. The effect of starvation on web geometry in an orb-weaving spider. *Bulletin of the British Arachnological Society* **10**: 295-298.

Weissmann, M. 1987. Web-building and prey capture in two orb weavers, *M.Sc. thesis*, University of Oxford. England.

Weltzien, P.; Barth, F. G. 1991. Volumetric measurements do not demonstrate that the spider brain “central body” has a special role in web building. *Journal of Morphology* **208**: 91-98.

Witt, P. N. 1952. Ein einfaches prinzip zur deutung einiger proportionen im spinnennetz. *Behaviour* **4:** 172-189.

Witt, P N. 1971. Drugs alter web-building of spiders: A review and evaluation. *Behavioural Science* **16**: 98-112

Witt, P. N.; Reed, C. F. 1965. Spider-web building. *Science* **149**: 1190-1197

Witt, P. N.; Reed, C. F.; Peakall, D. B. *A spider’s web: problems in regulatory biology*. Springer Verlag, Berlin. 1968.

Wolff, D.; Hempel, U. 1951. Versuche über die beeinflussung des netzbaues von *Zilla x-notata* durch pervitin, scopolamin und strychnin. *Zeitschrift für Vergleichende Physiologie* **33**: 497-528.

Zschokke, S. 1993. The influence of the auxiliary spiral on the capture spiral in *Araneus diadematus* Clerk (Araneidae). *Bulletins of the British Arachnological Society* **9**: 169-173.

Zschokke, S. 1994. Web construction behaviour of the orb-weaving spider *Araneus diadematus* Cl. *Ph.D. thesis*. Faculty of Philosophy and Science, University of Basel, Switzerland.

Zschokke, S.; Vollrath, F. 1995. Unfreezing the behaviour of two orb spiders. *Physiology and Behavior* **58:** 1167-1173.

Chapter 5: General discussion

Elaboration

Like most research in the life sciences, behavioural studies rely heavily on the concept of trial and error. What appears to be a perfectly simple and straightforward experiment, may in fact turn out to be impossible due to the constraints set by the co-operation of the experimental animal. In fact, when designing an experiment in ethology the choice of the right model organism is often vital (Martin and Bateson 1993). In this master project the choice of the orb-weaver *Araneus diadematus* was obvious since a survey of the literature revealed this to be the most popular orb-weaver for similar studies. However, this choice was also supported by evidence from other spiders in the lab. I kept several other species of orb-weavers in the lab to assess their suitability in studies dealing with web-building behaviour. Out of 37 *Lariniodes cornutus (*fam. Araneidae*)* kept in frames for more than a week in the lab under standard conditions, 21 built webs but only 5 individuals built 3 or more webs. *Nephila edulis* (fam. Tetragnathidae) taken from a laboratory stock housed in the spider room at the University of Aarhus showed an acceptable web-building frequency. However, *Nephila* spiders do not remove the auxiliary spiral from their webs (Landolfa and Barth 1996). This together with more asymmetrical and fine-meshed webs makes them less suited for web-analysis. A possible alternative to *Araneus diadematus* is the slightly smaller *Zygiella x-notata* (fam. Araneidae), which has also been used extensively in orb-weaver studies. However, *Zygiella x-notata* exhibits a slightly smaller web-building frequency. Late instar juvenile *Zygiella x-notata* has a web-building frequency around 60%-70% (Mayntz in prep) compared to late instar juvenile *Araneus diadematus* with a web-building frequency of around 80% (Vollrath and Samu 1997; this thesis chapter 4).

In chapter 2 it was convincingly shown that temperature had a significant effect on both the spider's capture time and the escape time of the prey. However, only one prey type, the fruit fly *Drosophila melanogaster*, was used and since both prey size and activity level is important for the escape time (Nentwig 1982), it would be of interest to see if other prey types exhibited a different temperature dependence in escape time. To determine any effect in the wild of the observed temperature dependence of the predator-prey system, it would, furthermore, be necessary to correlate the lab experiments with actual observations in the field. These could include observations of type and size of potential prey active at different temperatures and counting of intercepted prey in either real or artificial webs (see Pasquet and Leborgne (1990) and Nentwig (1983) for useful approaches). Furthermore, since *Araneus diadematus* usually builds its web in the early morning (Spronk 1935), more data is needed on the correlation between temperature at the time of web-building and temperature later in the day when most prey is caught. In the current study the capture time of the spider and the escape time of the prey was measured separately in two different experiments. This is the only sound method to acquire accurate estimates of these two factors, however, it is conceivable that the behaviour of the spider influences the escape time of the prey. When the web intercepts a prey, the spider sometimes jerks the web violently presumably to determine the exact location of the prey (Klärner and Barth 1982; Suter 1978). However, this jerking is sometimes so strong that the prey is moved visibly causing it either to escape rapidly or become attached to more threads (pers. obs.). It is therefore possible that web-jerking is also used to ensure that the prey is retained long enough for the spider to reach it.

The experiment about the effects of temperature on web-geometry and web-building behaviour, described in chapter 3, failed to find any on the former, but clearly showed that a decrease in temperature leads to a decrease in building speed. However, due to the relatively low sample size in this experiment it is difficult to come up with a rigid conclusion from the results. The main reason for the limited sample size was the unexpected low web-building frequency in the climate chamber. The cause of which remains a puzzle. As the frames were enclosed between plastic sheets, the air current in the chamber should not have any effect. Attempts were made to place the frames on vibration damping pads, but this did not result in any visible increase in web-building frequency. It is possible that the red cellophane in the climate cabinet allowed other wave-lengths to pass through thereby preventing any totally dark periods. As *Araneus diadematus* has the highest web-building frequency during dark periods (Ramousse and LeGuelte 1979; Ramousse and Davis 1976), this might explain some of the observed decrease in web-building frequency. The lack of temperature fluctuations in the climate chamber may also play a role, as observations indicate that *Araneus diadematus* preferably builds web during temperature minima (Witt 1963; Spronk 1935). Another hitherto neglected environmental variable, which might be important, is barometric pressure. There is evidence that the web size of another closely related orb-weaver *Araneus quadratus* is influenced by barometric pressure (Ammitzbo 1988). As barometric pressure in early morning can be used to predict the weather later in the day, it would be reasonably to assume that this variable would have an influence on web-building frequency. Barometric pressure in the air-tight climate cabinet could very well be different from the outside pressure. As noted earlier, observations indicate that the time of web-building is determined, together with the light/darkness cycle, by changes in temperature more than by the actual temperature itself (Witt 1963; Spronk 1935). It would therefore be interesting to compare experiments, as the one done in chapter 2, with lab experiments where the spiders were exposed to daily fluctuations in temperature. This would allow comparisons between geometry of webs constructed at the same temperature, but under different regimes of either constant or fluctuating temperature.

In the last experiment on the effects of drugs on web-geometry and web-building behaviour (see chapter 4), it was found that both amphetamine and caffeine had severe effects whereas scopolamine had little effects. The number of drugs tested was limited to the three drugs mentioned, chosen because of their reported effects. However, nearly all drugs tested seem to affect the geometry of the webs (Witt and Reed 1965), so other drugs might result in different and equally interesting disruptions of the web-building programme. Based on the results obtained in this study, I would, though, prioritise more detailed studies with the neurotoxins used here over a wider selection of drugs. Especially more information concerning the dose-dependent effects of the neurotoxins would be informative, since several drugs show strong and even opposite effects depending on dosage (Witt and Reed 1965; Wolff and Hempel 1951). A wider approach to the behavioural effects of neurotoxins on orb-weaving needs to address the actual effects on the spider in more detail. This could be done by conducting an experiment similar to the one described in chapter two. The predatory behaviour of drugged spiders in normal webs could be tested to generate more information as to whether primarily the motor or the sensory system was affected by the neurotoxin in question. Another interesting aspect is the effect of neurotoxins on memory in the orb-weaver. Scopolamine and caffeine for instance are known to affect memory and retention in various invertebrates (Fiorito et al. 1998; Dudai et al. 1986; Folkers and Spatz 1984). If a similar effect is found in spiders, the drug experiments could ultimately provide information about the role of short-term memory in web-building. Fortunately orb-spiders are quite suitable for these kinds of studies. Bays (1962) trained spiders to discern between two frequencies generated by a tuning fork. This experiment could be repeated using drugged and control spiders to see if the neurotoxins have a negative impact on learning and retention. Furthermore, *Araneus diadematus* exhibits several easily habituated traits. For instance, recently caught spiders show a high degree of protective behaviour when being handled (i.e. violently shaking in the hub thereby blurring the outline of the spider and dropping out of the hub), which almost totally disappears after some time in captivity (pers. obs.). A controlled experiment could be designed to reveal any difference between drugged and normal spiders in the number of trials necessary to habituate to disturbances. The closely related *Zygiella x-notata* is even better suited for memory studies since it does not sit in the hub, but builds a retreat where it returns promptly from every excursion. *Zygiella* has difficulties with relocating the retreat when the web is turned upside down, even if this happens before the spider leaves the retreat (LeGuelte 1969). The spider apparently primarily uses memory and past experience to relocate the hub and it would be interesting to see how neurotoxins would affect this. However, as indicated by a comparison between the effects of neurotoxins on juvenile *Araneus diadematus* obtained in this study and on adult *Araneus diadematus* and *Zygiella x-notata* from previous studies (Witt and Reed 1965), care needs to be taken when comparing results obtained from studies using different species or age-classes. In a study of the effects of amphetamine on different age classes of *Araneus diadematus*, Reed and co-workers (1982) found differences in sensitivity among the age-classes. The youngest animals were affected the least, however, this was possibly a matter of difference in body-mass and not age per say. The difference between species is emphasised by a trial experiment, where the sensitivity of alcohol was compared between juvenile *Araneus diadematus* and adult *Zygiella x-notata*, which revealed that *Zygiella x-notata* was the most sensitive of the two (Cecilie F. Holm unpublished). The possible differences between *Araneus diadematus* and *Zygiella x-*

notata in their responses to neurotoxins affecting the web-geometry, might arise from different web-building programmes. On the basis of observed differences between the two species in compensating for leg loss during web-building, it is likely they use different behavioural rules for web-construction (Weissmann and Vollrath unpublished, Vollrath 1990). Lastly radioactive labelling of drugs could productively be employed as a supplement. This method makes it possible to locate specific target sites of the neurotoxins in the spider CNS. Ultimately this approach could yield important information about which parts of the spider brain is important for various aspects of web-building.

Throughout this master study both the behaviour of the spider and the characteristics of the web have been dealt with. However, the actual building material of the web, the spider silk, has been virtually ignored. The physical properties of the individual silk threads and the tension of these are important for generating vibrations and for absorbing the kinetic energy of the incoming prey (Köhler and Vollrath 1995; Eberhard 1981). Several factors affect the physical properties of silk including water content in the air (Edmonds and Vollrath 1992; Work 1981) and anaesthetising of the spider with CO_2 and N_2 (Madsen and Vollrath 2000). It is therefore likely that the neurotoxins used here may have some impact on the properties of the silk. Especially as the experiment with anaesthetising the spiders indicate that CNS control of the spinning apparatus determines the silk properties (Madsen and Vollrath 2000). However, the neurotoxins could also have more direct effects on the silk itself. Cholinergic drugs like physostigmine increases silk synthesis (Peakall 1964). Although, Peakall (1964) found no effects of anticholinergic drugs on silk synthesis, drugs might have an effect on the physical properties of the silk. In future studies, it would therefore be useful to take thread samples from the web and compare the physical properties of these between drug webs and normal webs. There could conceivably be an interplay between the effects of neurotoxins on the actual web-building behaviour and its indirect effects on behaviour through feedback from changes in silk properties.

Conclusion

In this thesis, it was shown that changes in both external and internal factors influenced the web-building behaviour of the spider *Araneus diadematus.* However, whereas the application of neurotoxins, which was used to disrupt the CNS of the spiders, in most cases proved to have significant effects on the geometry of the orb-web, no such effects of the external factor, in this case temperature, was found. To allow for an eco-ethological approach the effects of temperature were investigated for both web-building and predatory behaviour. In the latter, temperature had profound effects on both reaction time, orientation time and prey capture speed of the spider resulting in an overall decrease in capture time with higher temperatures. Combined with escape times of a representative prey species from empty webs, the results suggested that the capture efficiency of the spider declined at very high temperatures. A similar effect of temperature was seen on the web-building speed of the spiders, which increased significantly with higher temperatures. However, the spiders apparently did not compensate by decreasing web-size or increasing mesh size for the longer time required to build a web at lower temperatures, although the low sample size prevented firm conclusions.

In the second part of the thesis the influence of an internal factor, the change in the state of the central nervous system, was investigated by administration of three potent neurotoxins. Both amphetamine and caffeine exerted strong, but different, effects on the web-geometry and web-building behaviour. Scopolamine was tested at two different concentrations, and although none had significant effects on web-geometry there was a trend towards a higher degree of disturbance with higher concentrations. The high concentration of scopolamine, furthermore, resulted in a significant decrease in web-building frequency. In conclusion this study revealed that application of neurotoxins to orb-weavers is a promising method to increase our knowledge of the structure of the web-building programme. The web-building behaviour and the resultant orb-web have the potential to become an ideal model system for studies in invertebrate behaviour. Especially, an integrative approach incorporating elements of neurobiology and computer simulations might be a rewarding path for future research.

References

Ammitzbo, A. Fouragering hos *Araneus quadratus* Clerk. *M.Sc. thesis*. University of Aarhus. Aarhus 1988.

Bays, S. M. 1962. A study of the training possibilities of *Araneus diadematus* Cl. *Experientia* **18**: 423-434.

Dudai, Y.; Buxbaum, J.; Corfas, G.; Orgad, S.; Segal, D.; Sher, B.; Uzzan, A.; Zvi, S. 1986. Defective cAMP metabolism and defective memory in Drosophila. *Acta Biochem. Biophys. Hung.* **21**: 177-192.

Eberhard, W. G. 1981. Construction behaviour and the distribution of tensions in orb-webs. *Bulletins of the British Arachnological Society* **5**: 189-204.

Edmonds, D. T.; Vollrath, F. 1992. The contribution of atmospheric water vapour to the formation and efficiency of a spider's capture web. *Proceedings of the Royal Society of London. Series B.* **248:** 145-148.

Fiorito, G.; Agnisola, C.; d'Addio, M.; Valanzano, A.; Calamandrei, G. 1998. Scopolamine impairs memory recall in *Octopus vulgaris*. *Neuroscience Letters* **253:** 87-90.

Folkers, E.; Spatz, H. C. 1984. Visual learning performance of *Drosophila melanogaster* is altered by neuropharmaca affecting phosphodiesterase and acetylcholine transmission. *Journal of Insect Physiology* **30**: 957-965.

Klärner, D.; Barth, F. G. 1982. Vibratory signals and prey capture in orb-weaving spiders (*Zygiella x-notata*, *Nephila clavipes*; Araneidae). *Journal of Comparative Physiology A* **148**: 445-455.

Köhler, T.; Vollrath, F. 1995. Thread biomechanics in two orb-weaving spiders *Araneus diadematus* (Araneae, Araneidae) and *Uloborus walckenaerius* (Araneae, Uloboridae). *The Journal of Experimental Zoology* **27**: 1-17.

Landolfa, M. A.; Barth, F. G. 1996. Vibrations in the orb web of the spider *Nephila clavipes*: cues for discrimination and orientation. *Journal of Comparative Physiology A*. **179**: 493-508.

LeGuelte, L. 1969. Learning in spiders. *American Zoologist* **9**: 145-152

Madsen, B.; Vollrath, F. 2000. Mechanics and morphology of silk drawn from anesthesized spiders. *Naturwissenschaften* **87**: 148-153.

Martin, P.; Bateson, P. *Measuring behaviour: an introductory guide 2nd Ed.* Cambridge University Press. 1993.

Nentwig, W. 1982. Why do only certain insects escape from a spider's web? *Oecologia* **53**: 412-417.

Nentwig, W. 1983. The non-filter function of orb webs in spiders. *Oecologia* **58**: 418-420.

Pasquet, A.; Leborgne, R. 1990. Prey capture efficiency and prey selection from insects intercepted by trap in four orb-weaving spider species. *Acta Æcologica* **11**: 512-523.

Peakall, D. B. 1964. Effects of cholinergic and anticholinergic drugs on the synthesis if silk fibroins of spiders. *Comparative Biochemistry and Physiology* **12**: 465-470.

Ramousse, R.; Davis, F. 1976. Web-building time in a spider: preliminary applications of ultrasonic detection. *Physiology and Behavior* **17**: 997-1000.

Ramousse, R.; LeGuelte, L. 1979. Relations spatio-temporelles dans le comportement constructeur chez l'Epeire diadème. *Revue Arachnologique* **2**: 183-192.

Reed, C. F.; Witt, P.N.; Scarboro, M. B. 1982. Maturation and *d*-amphetamine – induced changes in web building. *Developmental Psychobiology* **15**: 61-70.

Spronk, F. 1935. Die abhängigkeit der netsbauzeiten der radnetz-spinnen *Epeira diademata* und *Zilla x-notata* von verschiedenen aussenbedingungen. *Zeitschrift für Vergleichende Physiologie.* **C22**: 604-613.

Suter, E. B. 1978. *Cyclosa turbinata* (Araneae, Araneidae): Prey discrimination via web-borne vibrations. *Behavioral Ecology and Sociobiology* **3**: 283-296.

Vollrath, F. 1990. Leg regeneration in web-spiders and its implication for orb-weaver phylogeny. *Bulletins of the British Arachnologicl Society* **8**: 177-184.

Vollrath, F.; Samu, F. 1997. The effect of starvation on web geometry in an orb-weaving spider. *Bulletin of the British Arachnological Society* **10**: 295-298.

Witt, P. N. 1963. Environment in relation to behavior of spiders. *Archives of Environmental Health* **7**: 10-18.

Witt, P. N.; Reed, C. F. 1965. Spider-web building. *Science* **149**: 1190-1197

Wolff, D.; Hempel, U. 1951. Versuche über die beeinflussung des netzbaues von *Zilla x-*

notata durch pervitin, scopolamin und strychnin. *Zeitschrift für Vergleichende Physiologie* **33**: 497-528.

Work, R. W. 1981. A Comparative study of the super-contraction of major ampullate silk fibers of orb-web-building spiders (Araneae). *Journal of Arachnology* **9**: 299-308.