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Natural enemies of oleander aphid, *Aphis nerii* B. de F. (Hom., Aphididae), effective factors in attraction of coccinellids and population fluctuation of oleander aphid in Shiraz and Ahvaz (Iran)

Maryam ALEOSFOOR & Mohammad Saeed MOSSADEGH

Abstract

During Mar. 2001 to Aug. 2013, *Aphis nerii* B. de F. and its natural enemies were studied in Shiraz and Ahvaz. At Shiraz condition, the aphid fed and produced generations on *Nerium oleander* whole the year but in Ahvaz when the temperature reached to 38- 40˚C, they disappeared. It's probable that *A. nerii* migrates to the cold valleys of mountainous areas in the north of Ahvaz.

Altogether, 4 groups of natural enemies were collected and identified. They include 15 species of Coccinellidae, 5 species of Syrphidae, one species of Chrysopidae and one species of Braconidae. Studding on different factors that seem to be affected in attraction of four aphidophagous coccinellids *Coccinella septempunctata*, *Hippodamia variegata*, *Exochomus nigromaculatus* and *Ex. nigripennis* showed that neither honeydew nor leaf and Distillate of plant are sufficient for attraction of coccinellids. Thus In addition, the presence of oleander aphid colonies, complexes of factors are necessary to attract the coccinellids.

Key words: natural enemies, *Nerium oleander*, Coccinellidae, Syrphidae, Chrysopidae.

Zusammenfassung

Während März 2001 bis August 2013 wurden *Aphis nerii* B. de F. und ihre natürlichen Feinde in Shiraz und Ahvaz studiert. Unter den Bedingungen in Shiraz, leben und produzieren die Blattlause das ganze Jahr ihre Generationen auf *Nerium oleander*. Jedoch in Ahvaz, wo die Temperatur 38-40°C erreicht, verschwanden sie. Es ist anzunehmen, dass *A. nerii* sich in die kalten Täler der Bergregionen im Norden von Ahvaz zurückzieht.

Insgesamt wurden 4 Gruppen von natürlichen Feinde gesammelt und identifiziert. Sie umfassen 15 Arten der Coccinellidae, 5 Arten der Syrphidae, eine Spezies der Chrysopidae und eine Art der Braconidae. Nach Untersuchungen von verschiedenen Faktoren scheint die Anlockung von vier aphidophagen Coccinelliden: *Coccinella septempunctata*, *Hippodamia variegata*, *Exochomus nigromaculatus* und *Ex. nigripennis* zu zeigen, dass weder Honigtau noch Absonderungen auf Blättern von Pflanzen ausreichend attraktiv für Coccinelliden sind. So sind zusätzlich die Anwesenheit von Oleander-Blattlaus-Kolonien und verschiedene Faktoren erforderlich, um Coccinelliden anzulocken.

Introduction

The genus *N. oleander*, belonging to the family Apocynaceae, is a highly toxic evergreen ornamental shrub. It is so widely cultivated that no precise region of origin has been identified, though Mediterranean, Asia and Iran has been suggested (SABETI 1976; KHAVARI & MOOSAVI 1999). Oleander is tolerant of poor soils and drought and will tolerate occasional light frost down to −10°C. The subspecies *'N. indicum* Mill' and *N. oleander* L. are widely dispersed throughout Iran. Three distinct phenotypes of *N. oleander* including *Oleander album*, *N. oleander carnum* and *N. oleander roseum* can be found in the urban landscapes of Shiraz and Ahvaz. These phenotypes are white, red and pink in flower colour, respectively (KHAVARI & MOOSAVI 1999). Even though the sap-bearing segments of this plant are toxic and cause cardiovascular and gastrointestinal disorders in many organisms (BEHDAD 1996; MOODI & MOSSADEGH 2000; ABDUL-SATAR et al. 1998), some invertebrates like *Oleander* aphid, *Aphis nerii* B. de F., are known to be unaffected by oleander toxins, and feed on the plant (HODJAT 1993). The oleander aphid, which is sometimes called Milkweed aphid, is a serious pest of ornamental plants from family Apocynaceae and Asclepiadaceae (HALL & EHLER 1980; JOHNSON & LYON 1988). This aphid sucks the plant sap and gives off tremendous amounts of honeydew. Furthermore, it raises deformity in leaves and causes stunted growth (BEHDAD 1988; HODJAT 1993).

This aphid has a worldwide dispersion and is abundantly found in temperate and tropical regions. It was reported by Rezvani in 1986 from Tehran, Iran (REZVANI & PARVIZI 1989). Little research has been conducted on the Milkweed aphid and its enemies throughout the world. However, ABDUL-SATAR et al. (1998) have assessed its natural enemies in Baghdad, Iraq, while Takada & Sugimoto (TAKADA & SUGIMOTO 1994) have studied the aphid's life cycle and its natural enemies in Kyoto, Japan. As mentioned earlier, Iran is thought to be one of the origins of this plant; therefore, the natural enemies

of this pest can be found indigenously in Iran. Furthermore, a decisive incursion into the knowledge of the Milkweed aphid and its natural enemies has not been carried out in Iran, although several coccinellid species have been collected from this plant and subsequently reported (KOOHPAYEZADEH & MOSSADEGH 1993; MONTAZRRI & MOSSADEGH 2000; MOODI & MOSSADEGH 2000). Due to the increasing interest in the replacement of biological control with chemical one and the importance of *N. oleander* as a dry-tolerance ornamental plant, this study was designed.

Material and methods

Population fluctuation of *A. nerii*

In order to assess the population fluctuations of the Milkweed aphid, sampling from *N. oleander* bushes were done weekly from April 2001 to August 2013 in Shiraz and Ahvaz. The role of temperature and relative humidity in determining the aphid population fluctuation was studied here. So, a total number of 476 *N. oleander* bushes were sampled in Shiraz and Ahvaz and checked for aphid and its natural enemies. The collected samples were taken to the germinator in enclosed plastic containers (35*24*9cm) that had 4 holes (2.5 cm diameters) covered up in white cloth at their lid and kept under 25±1 ºC, 75±5 RH and a photoperiod of 14:10 lightness: darkness conditions. Some winged-aphids were removed from the leaves and branches with fine brushes. They were conserved in McCarthy jars containing 75% alcohol and were kept there until the preparation of microscope slides.

Natural enemies' collection

The aphid's natural enemies were also collected. Eggs, larvae and pupae of coccinellids, syrphids and mummy aphids were collected and preserved in germinator under the aforementioned conditions until the adult insects emerged. Microscope slides of genitalia and other parts of the natural enemies were prepared for identification purposes. Collected samples were identified with the help of Museum of Entomology, Shahid Chamran Ahvaz University and relevant keys. The reaction of 100 aphids in response to the coccinellid offensive was observed under binocular viewing.

Coccinellids rearing for olfactory experiments

These experiments were conducted in the Research Laboratory, Department of Entomology, at Shahid Chamran Ahvaz University, Iran. To obtain eggs and larvae, the newly emerged adult of four coccinellid species (*H. variegata*, *C. septempunctata*, *Ex. nigripennis* and *Ex. nigromaculatus*) were collected from the oleander bushes and released in pairs and confined inside plastic containers (35*24*9cm) at suitable temperature 25±1 ºC, 75±5 RH and a photoperiod of 14:10 lightness: darkness. Each container was covered with muslin cloth. Coccinellids accumulated were fed with oleander aphid, reared on *Nerium oleander*.

Olfactometer experiments

The adult coccinellids were 24 and 72 hr days old and tested individually. All coccinellids provided only with water form emergence to the behavioural test and none of them had any contact with the tested odour sources previous to the experiment. A maximum of 5 min was given to each coccinellids to make its final choice. After testing 5 insects, the whole apparatus was rotated 90º and after testing each coccinellid, it was cleaned with 95% ethanol.

To start a measurement, one coccinellid was transferred from the container and placed in the frontal cavity of olfactometer (Fig. 1). We tested the attractiveness of oleander aphid, honey dew, healthy leaves, honey dew-stained paper, normal paper, aphid-bearing leaves + honeydew+ plant sap to the coccinellids, i.e. whether the odour sources were attractive. The small fraction of females that did not enter the arms within five minutes were considered as non responders and removed from the experiment. This experiment was conducted with the deployment of four coccinellid species, *H. variegata*, *C. septempunctata*, *Ex. nigripennis*, *Ex. nigromaculatus* with the olfactometer (Fig. 1).

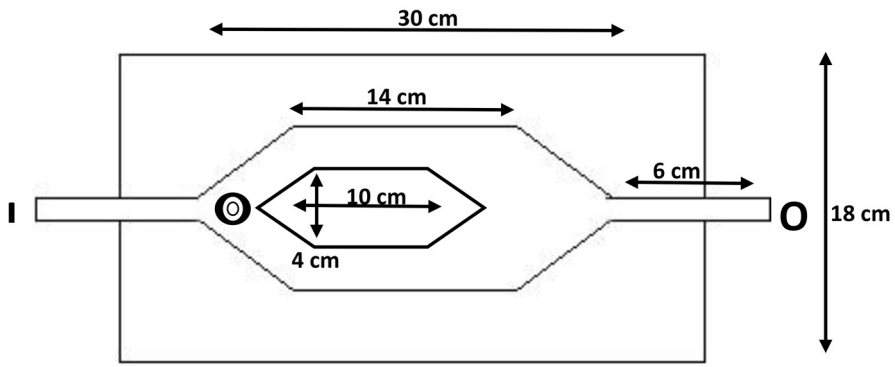


Fig. 1: Dimensions and overall shape of olfactometer, I: air inlet, O: air outlet

In order to randomize possible side-effects on the preferences of coccinellids, odour sources were renewed for each replicate and their positions alternated between replicates. When a coccinellid exhibited a preference for an odour source, it was considered attractive. In the other hand, crossing the junction line for the first time is used to test whether a preference could be detected as soon as the females entered an arm or whether the females needed some exploration before displaying a preference. Ten replicates were performed on a given experimental day and the experiments were repeated sixty times for each coccinellid species.

Furthermore, for eliminating the effect of light, all the mentioned experiments were conducted under a red light situated 0.5 metres above the olfactometer.

Statistics

The data were normally distributed, so we performed a T TEST (SPSS Inc. Released, 2012). charts were drawn by Excel (2007) software.

Results

Studying the population fluctuation of *A. nerii*

Under the suitable climatic conditions of Shiraz, this aphid was observed on *N. oleander* all year round. However, its population density was influenced by seasonal temperatures. March through June, was the period of time with the maximum number of aphid individuals, whereas, in July, January and December saw the least. On the contrary, due to the unfavourable climatic conditions in Ahvaz, the aphid was only observed in April, May, early June, late February and March. Its absence on *N. oleander* persisted through the remaining months (Fig. 2).

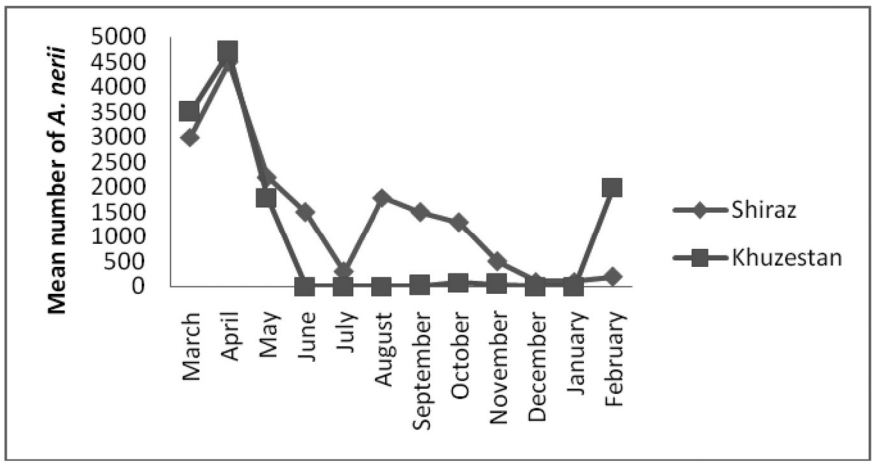


Fig. 2: Mean number of *A. nerii* on 476 *N. oleander* bushes in Shiraz and Ahvaz during 2001-2013.

Studding on the natural enemies of the *A. nerii*

A collection of 15 coccinellid species belonging to 8 genera, 5 species of Syrphids, 1 species of Chrysopidae and a parasitoid wasp were gathered and identified as explained in table 1.

Tab. 1. Natural enemies of *A. nerii* in Shiraz and Ahvaz during 2001-2013.

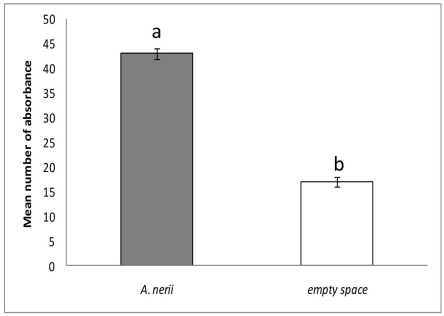
Species	Family	Location
Coccinella septempunctata L.	Coccinellidae	Shiraz and Ahvaz
C. undecimpunctata L.	Coccinellidae	Shiraz and Ahvaz
Hippodamia variegata GOEZE	Coccinellidae	Shiraz and Ahvaz
Oenopia oncina OLIVER	Coccinellidae	Shiraz
O. conglobata L.	Coccinellidae	Shiraz
Adalia bipunctata L.	Coccinellidae	Shiraz and Ahvaz
Cheilomenes sexmaculata F.	Coccinellidae	Shiraz and Ahvaz
Exochomus quadripustulatus L.	Coccinellidae	Shiraz
Ex. nigromaculatus GOEZE	Coccinellidae	Shiraz
Ex. nigripennis ERICHSON	Coccinellidae	Shiraz and Ahvaz
Ex. undulatus WEISE	Coccinellidae	Shiraz
Ex. pubscens KUSTER	Coccinellidae	Shiraz
Scymnus (*Pullus*) *syriacus* MARSEUL	Coccinellidae	Shiraz
S. mongolicus WEISE	Coccinellidae	Shiraz
Nephus ulbrichi FURSCH	Coccinellidae	Shiraz
Ischiodon aegyptius (W.)	Syrphidae	Shiraz and Ahvaz
Eupeodes nuba (W.)	Syrphidae	Ahvaz
Scaeva albomaculata M.	Syrphidae	Ahvaz
Sphaerophoria bengalensis M.	Syrphidae	Shiraz and Ahvaz
Paragus compeditus W.	Syrphidae	Ahvaz
Chrysoperla carnea S.	Chrysopidae	Shiraz and Ahvaz
Lysiphlebus testaceipes (CRESSON)	Braconidae	Shiraz and Ahvaz

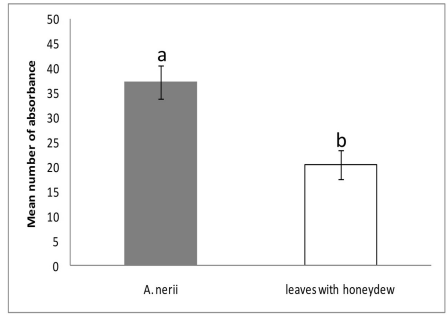
The aphids' reactive behaviour against the coccinellid offensive

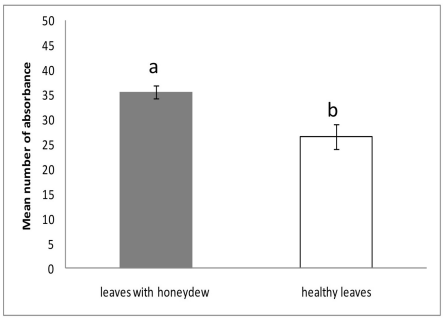
Studying the reaction of oleander aphid against predators attack showed that, after an aphid being caught, a fraction of about 40% of the total aphid population began to stand on their frontal and medial legs and heaved their hind legs and abdomen from the surface. They frequently relocated their hind legs and abdomen towards the location where they received the alarm pheromone. This act was performed communally, rhythmically and simultaneously. About 35% of the aphid population withdrew their mouth parts from the Phloem and escaped.

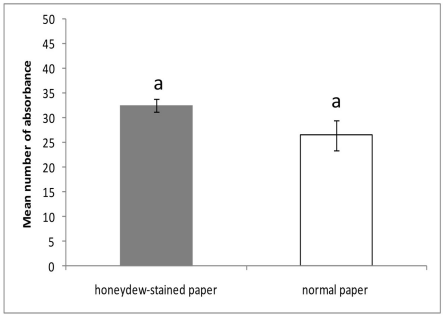
Results of experiments with the olfactometer

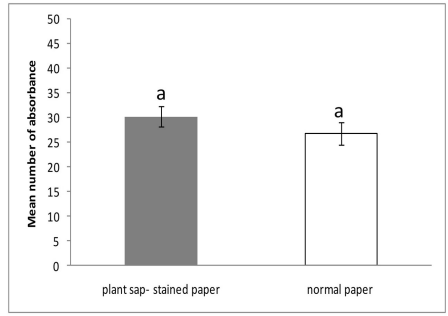
1. No significant difference was observed among the coccinellid species used in the entirety of the experiments ($p>0.05$).
2. Whenever the oleander aphids were located on one side and the other side was empty, a significant difference were seen between the percentages of instances that the four species of coccinellids moved towards the aphids instead of moving to empty space ($T=17.02$, $p=0.0001$). In other words, the coccinellids easily distinguished the aphid-bearing leaves and headed for it in 75% of the occasions (Fig. 3).











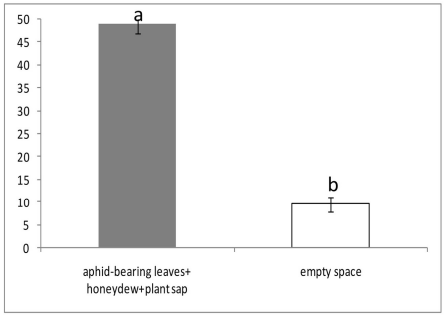


Fig. 3: Results of experiments performed with four species of coccinellids, *H. variegate*, *C. septempunctata*, *Ex. nigripennis*, *Ex. nigromaculatus*, in olfactometer. Different letters indicate significant differences at 95% confidence level.

1. At a time when the Milkweed aphid was situated on one side and the leaves with honeydew were on the other, significant difference was seen in the coccinellids' choice of movement towards the olfactometer arms (T=3.78, p=0.01) (Fig. 3).
2. When healthy leaves and leaves with honeydew were placed on both sides of the device, the coccinellids select significantly more times the arm which is connected to honeydew in comparison to the other arm (T=3.14, p=0.026) (Fig. 3).
3. when normal leaves where placed in opposition to an empty space, the coccinellids couldn't make a concrete decision on their preference of taste; thus, an insignificance of difference was noted in this case (T=3.31, p=0.06) (Fig. 3).
4. The placement of honeydew-stained paper in opposition to normal paper resulted in an insignificant statistical difference in the coccinellids interest of movement (T=2.35, p=0.078) (Fig. 3).
5. On the occasion when paper that was stained with plant sap in opposition to the side of the device with normal paper, no significant statistical difference was observed in the instances of the coccinellids being attracted to either side (T=1.14, p=0.307) (Fig. 3).
6. Finally, the positioning of aphid-bearing leaves + honeydew + plant sap against an empty space of the device showed a significant inclination of the coccinellids towards the biomass (T=16.2, p=0.0001) to the extent that 90% of the coccinellids marched to that region (Fig. 3).

Discussion

The widespread role of landscape vegetation in enriching human living environments cannot be doubted. Air pollutions in urban locations, undesirable climates that percolate human livings and the less green sides of ultra-urban cities, all in all, can be controlled and held back by vegetation (THE MUNICIPALITY'S PARKS AND GREEN SPACES 1993). But unfortunately, toxic residuals are rising in ecosystems. From the equator to the poles, havens of life, especially urban residencies, have become the bearers of dangerous toxic materials. Problems like sterility, mutations in genes of organisms and hundreds of destructive effects are the consequences of applying such chemicals in the natural environment. Such issues have opened ground for the introduction and utilization of pest control methods without the involvement of excessive pesticides. The successful use of natural enemies in biological control methods is one of the most attended approaches in fighting pests, and ornamental plants are no exception (SMITH 1989).

Among the collected coccinellids in Shiraz and Ahvaz, 15 species were identified. The species *C. septempunctata*, *C. undecimpunctata*, *A. bipunctata*, *Ch. sexmaclatai* and *S. syriacus* were reported by KOOHPAYEZADEH & MOSSADEGH (1993), *H. variegata* by Kuhpayezadeh from Kerman and also by Montazeri from Golestan (MONTAZRRI M & MOSSADEGH 2000) and *Ex. nigripennis* by Khuhpayezadeh from Kerman & Moodi from south-east Khorasan (KOOHPAYEZADEH & MOSSADEGH 1993; MOODI & MOSSADEGH 2000). These coccinellids were collected from *N. oleander* and corresponded to the samples identified in this study. Furthermore, there are 7 species of coccinellids in the world that are known to be the natural enemies of this aphid (CARVERS 1989; GORDON

1985; PUGALENTHI & LIVINGSTONE 1997; TAKADA & SUGIMOTO 1994), three of which – *Ch. sexmaculata*, *H.variegata*, *C. undecimpunctata* – were the ones identified in this study. Other coccinellid species have been reported for the first time as natural enemies of *A. nerii* in the world.

This study showed that adult insects and larvae of three coccinellids – *C. septempunctata*, *H. variegata* and *A. bipunctata* – fed on the Milkweed aphid. On the contrary, HODEK (1973) quoted from Iperti that only *H. variegata* is able to feed on this aphid and the other two coccinellids are prone to toxicity upon their consumption of this aphid (HODEK 1973).

Apart from the coccinellids, 5 species of flower flies, one species of chrysopidae and one parasitoid wasp were collected in this survey. None of the identified Sirfid species have been reported as natural enemies of the Milkweed aphid. But the chrysopid and parasitoid wasp have been reported previously by Takadda (TAKADA & SUGIMOTO 1994).

The flow of the Milkweed aphid's life cycle in the favourable climatic conditions of Shiraz has caused the perpetual reproduction of coccinellids generations and has ultimately caused the increase in the population of these predators. In connection with this, some coccinellid species such as *C. septempunctata*, *C. undecimpunctata*, *H. variegata*, *Ex. nigripennis*, *Ex. quadripustulatus*, feed on this aphid for 5-7 months of the year and subsequently proceed to reproduction. On the other hand, in Ahvaz, with temperatures over 40°C, the aphids migrate to cooler environments, such as the Mountainous areas in the northern regions of the province, so the natural enemies have less time and opportunity to feed on this aphid. In any case and in both regions, we are able to reduce the aphid's population by rearing and releasing the natural enemies, especially in the early activity period of oleander aphid with out using insecticides. By this technique, environmental pollution will be prevented.

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Buchbesprechung

WEHNER R. & W. GEHRING: **Zoologie.** – Georg Thieme Verlag, Stuttgart-New York 2013, 25. Auflage, 792 S.

Mit dieser "Jubiläumsausgabe" folgt etwa 6 Jahre nach der erstmals großformatigen 24. Auflage die Neufassung des Zoologie-Klassikers, weiterhin aus der Feder von nur zwei Autoren. Die beiden in der Schweiz und Deutschland lehrenden Professoren Rüdiger Wehner und Walter Gehring bemühten sich, in den einzelnen Disziplinen die grundlegenden Fragestellungen und Konzepte herauszustellen und mit Forschungsbeispielen jeweils zu untermauern. Die vorliegende 25. Auflage wurde stark überarbeitet und aktualisiert; erstaunlich, dass sich der Gesamtumfang dabei eher deutlich verringerte (792 S. aktuell, 24. Aufl. 951 S.). Die Grundgliederung in 12 Kapitel wurde beibehalten und alle Kapitel sollten als selbstständige Texte gelesen werden können. Zahlreiche Querverweise helfen, sich beim Lesen auch jeweils anderen Themenkomplexen zuzuwenden und dort weitere Informationen zu finden. Ein besonderer Schwerpunkt wurde erneut auf das Kapitel "Vielfalt der Organismen" gelegt, also dem klassischen Systematischen Teil, der durchgehend einer phylogenetischen Systematik folgt, und etwa ein Fünftel des gesamten Textumfanges einnimmt.

Blätterst man einzelne Kapitel im Vergleich (zur 24. Aufl.) durch, wird man feststellen, dass sich eigentlich nicht sehr viel Neues getan hat, sieht man einmal vom moderneren Layout (Übersichten, Boxen) ab. Als Lehrender und auch Studierender kann man noch gut mit der 24. Auflage weiter arbeiten, was letztendlich für die beeindruckende Aktualität dieses Werkes spricht.

Der "Wehner, Gehring" wird DAS Zoologie-Lehrbuch im deutschsprachigen Raum bleiben, weil dieses Buch alle Teildisziplinen (Zellbiologie, Genetik, Neurobiologie, Ethologie, Ökologie, Evolution und Systematik) der Zoologie umfasst.

R. Gerstmeier

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