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Phenotypic variation in dark coloration in *Pityohyphantes phrygianus* (C. L. Koch) (Araneae: Linyphiidae)

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Summary

A phenotypic variation in dark coloration was found in a population of *Pityohyphantes phrygianus* (C. L. Koch) in SW Sweden. The specimens varied in colour from a pale phenotype, with little or no dark coloration, through intermediate varieties, to a melanic phenotype. Such phenotypes were distinguishable in adults as well as in juveniles of all stages. Subadult specimens were collected in autumn and spring from the natural population. The samples showed that the amount of dark coloration varies continuously among the specimens. Matings performed showed no reproductive constraints between different phenotypes. The results imply that *P. palilis* (L. Koch) is probably a melanic variety of *P. phrygianus*. It is suggested that the variation may be maintained by balancing selective forces.

Introduction

Pityohyphantes phrygianus (C. L. Koch) is a common spider on spruce (*Picea abies* (L.) Karst.) in northern Europe, but it is also found on pine (*Pinus silvestris* L.) (Palmgren, 1972). The life-cycle is biennial in southern Sweden and individuals pass their first winter as young juveniles and the second as subadults. All stages from spiderlings to adults could be found on spruce branches (Gunnarsson unpubl.). A considerable variation in the amount of dark coloration among the individuals was observed in a population in SW Sweden. The specimens varied from pale to melanic phenotypes.

In this paper I describe the colour variation observed in newly hatched spiderlings, juveniles and adults. A quantitative estimation of percentage dark coloration on abdomen and femur I was done on newly moulted adults. These were then used as reference specimens to assess the colour distribution among subadult specimens in samples from a natural population. I also performed mating experiments with the different phenotypes to test whether any reproductive barriers exist. The results support the suggestion by Simon (1929) that *P. palilis* (L. Koch) is a melanic variety of *P. phrygianus*. I also discuss some possible reasons for the maintenance of the colour variation in the natural population.

Material and methods

I collected the spiders in a vast coniferous forest east of Göteborg in SW Sweden. Most of the specimens used were taken about 3 km SE of the village Rävlanda and a few spiders were collected 2 km SW of the village Landvetter. In the first sampling area different samples were taken along a 2 km long route.

The samples were taken from spruce branches in different seasons. I enclosed the outer, needle-carrying, parts of spruce branches within a plastic sack (1.2 x 0.8 m). The branches were then shaken vigorously for about 30 seconds. Between 50 and 75 branches were used for each sample. In the laboratory I sorted out the *P. phrygianus* specimens from the samples and in most cases the spiders were immediately put into 70% ethanol. I recorded the coloration of the specimens within a month after preserving them, in order to avoid any bias from bleaching.

I measured the proportion of dark colours on femur I and the side of the abdomen in 20 newly moulted adult specimens collected in the autumn as subadults at Rävlanda and Landvetter. The spiders were kept at 4°C in the laboratory over the winter and they moulted to the adult stage after being fed on the vestigial-winged form of *Drosophila melanogaster* Meigen. The percentage of dark coloration in each spider was obtained by using a stereomicroscope with a drawing-mirror. I made a sketch of each femur I (ventral view) and abdomen (lateral view, spinners excluded) with the dark areas indicated, all darkish colours (black, grey and brown) being included. The dark areas were cut out from the sketch paper and weighed on a Mettler-balance. I selected eight spiders from the adults with a known percentage of dark coloration for use as reference specimens in comparisons with other spiders. The spiders used had 17%, 27%, 37%, 50%, 60%, 74%, 80%, and 90% dark coloration on the abdomen, respectively.

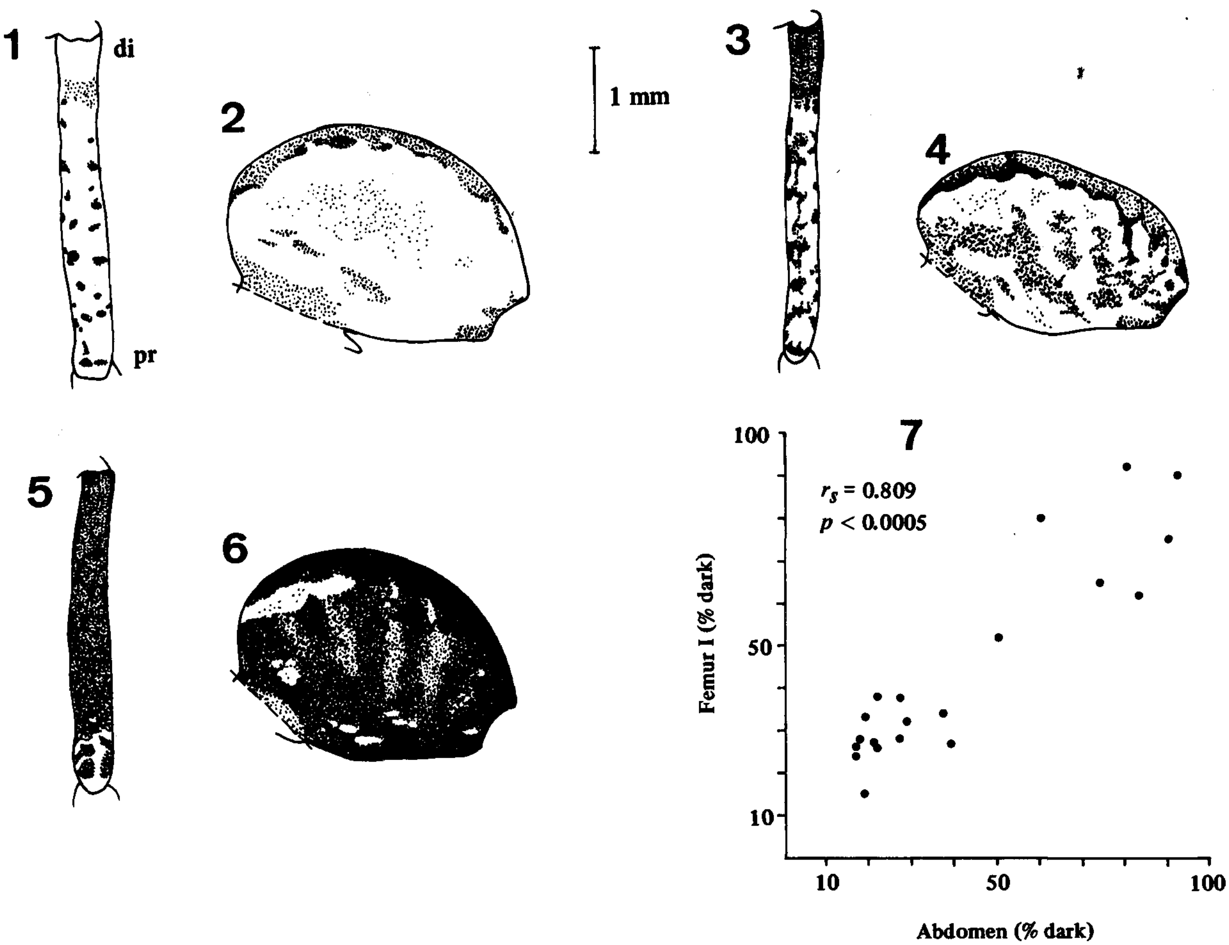
I performed mating experiments with the different phenotypes in the laboratory in May 1981. All specimens used were subadults when collected. Females in separate 500 ml plastic vials produced egg sacs after each was mated with a single male. Spiderlings emerged from the sacs and after a few days they were put into 70% ethanol.

Results

Adults

A phenotypic variation in dark coloration was observed among the adult spiders studied (Figs. 1-6). All adult individuals had a dorsal folium on the abdomen. This varied in colour, from greyish with irregular white spots and an incomplete brown border in pale specimens, to completely black in some melanic specimens. In intermediate spiders the folium was usually brown or dark brown with a dark, almost black border. The carapace had a forked, dark marking which varied in intensity among the individuals.

The lateral sides of the abdomen and the legs showed wide variation in amount of dark coloration (Figs. 2, 4, 6). The sides of the abdomen had a whitish ground colour but in some specimens the



Figs. 1-7: Variation in dark coloration in adult *P. phrygianus*. Ventral view of femur I and left side of abdomen (spinners excluded) are shown. Distal end of femur denoted by di, proximal end by pr. Hairs and spines omitted. Three main phenotypes on a continuous trait are shown: **1, 2** Pale; **3, 4** Intermediate; **5, 6** Melanic; **7** Correlation between percentage dark coloration on femur I and abdomen.

colour had a pale yellow tone. There were often reticulated areas where the underlying greyish colour appeared, and there were a varying number of dark brown or black longitudinal patches. The femora had a light yellow ground colour with black or dark grey spots or areas (Figs. 1, 3, 5). The proportion of dark coloration covering the lateral side of the abdomen varied between 17% and 92%, and on the ventral side of femur I it varied between 15% and 92%. The percentages of dark coloration on femur I and the side of the abdomen are highly correlated (Fig. 7; r_S = 0.809, $p < 0.0005$, Spearman rank correlation test). This therefore indicates that there is a similarity in the amount of pigmentation on different parts of the spider.

This description of the variation in dark coloration applies to newly moulted adults as well as to subadults. Adults of some age have their abdomen either distended (females) or shrunken (males), but when taking this into consideration it is possible to use the above description for "old" adults.

Juveniles

Spiderlings just emerged from the egg sac varied from pale specimens to specimens with distinct dark markings. For convenience I classified them into three groups (Figs. 8-10): (i) Specimens with a light brownish marking dorsally on the abdomen and sometimes with an incomplete triangular, dark patch forming the anterior part of this marking; (ii) Specimens with a brown marking dorsally on the abdomen and a complete dark patch; (iii) Specimens with a black marking dorsally and several black patches laterally on the abdomen. The carapace marking also varied in intensity and area covered. About 5% of the specimens investigated could be recognized as intermediates between the three categories, so most specimens fall into the three classes as described above. I have observed specimens of these three categories among spiderlings emerged from egg sacs laid by females in the laboratory as well as from egg sacs collected in the wild population.

Juveniles collected just after their first winter show a similar type of variation in dark coloration (Figs. 11-13) as those stages (spiderlings, subadults, adults) described above. I have not been able to follow growing individuals from egg-hatching through to the adult stage because of great losses among young juveniles kept in captivity. Studies in progress indicate that juveniles retain about the same proportion of dark coloration when moulting (pers. obs.), and no strong environmental impact on the expression of the phenotypes has been observed.

Variation in a natural population

I studied the phenotypic variation among subadults in a natural population near Rävlanda by taking three samples during a year. The collected spiders were classified into 10%-intervals of dark coloration on the abdomen, by comparison with reference specimens (see Material and methods). The samples were taken from two cohorts; from the first cohort came one autumn (1982) and one spring (1983) sample, and from the second cohort came a single autumn (1983) sample. In total 235 subadult spiders were investigated; the pale individuals were the most frequent and only one or two melanics were collected per sample (Figs. 14-16). No obvious discontinuous distribution of the dark coloration was observed. The melanics seem to be extreme phenotypes on a continuous trait. An arbitrary classification, based on percentage of dark coloration, into three main phenotypes may be done; pale spiders with 10 to 29%, intermediates with 30 to 69% and melanic spiders with 70 to 100% dark coloration on the abdomen.

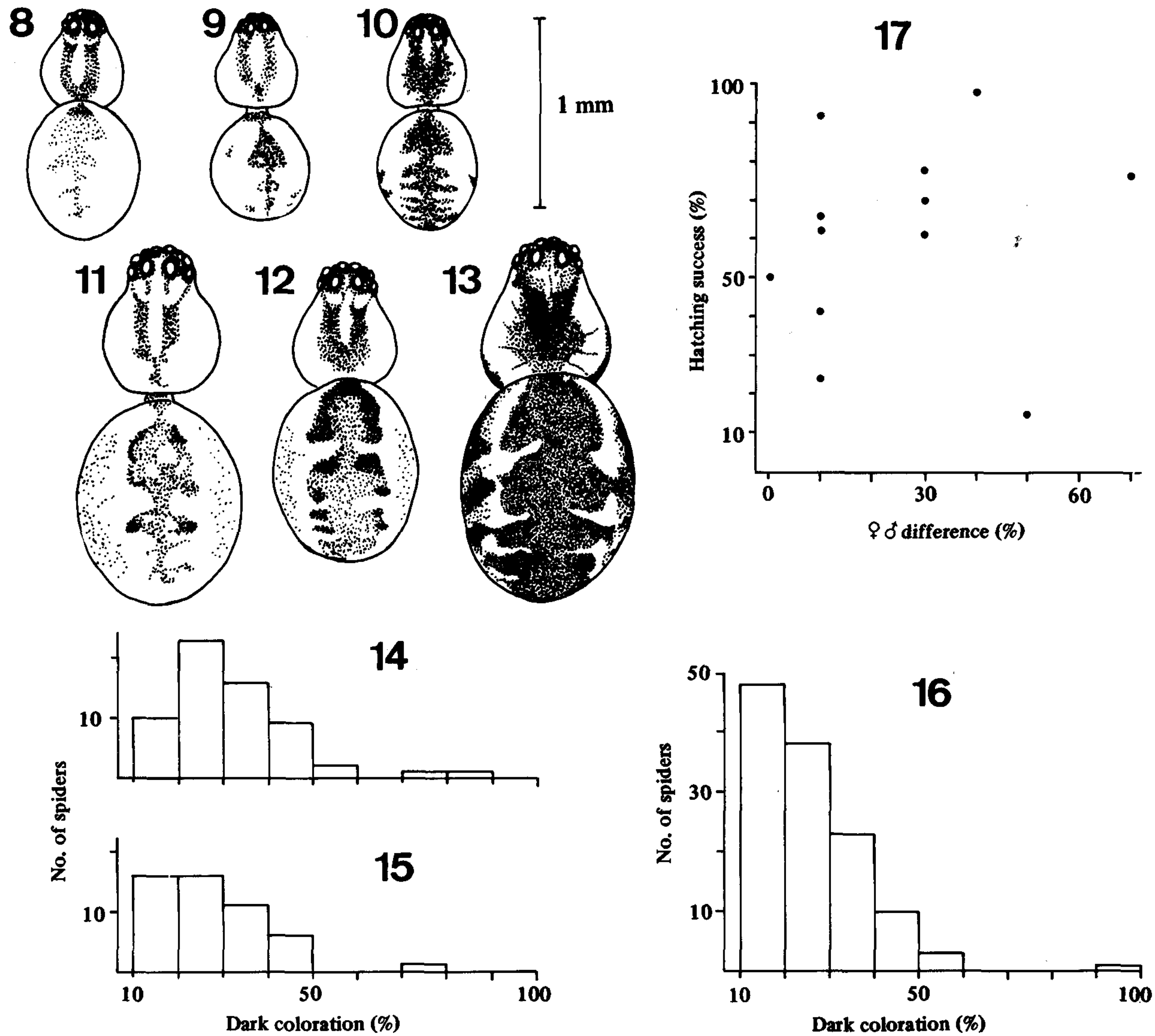
Matings between the phenotypes

To test whether there are different species among the phenotypes, eleven matings were performed. I used 21 previously unmated specimens; five females and three males of the pale phenotype, four females and five males of the intermediates, and two of each sex of the melanics. If the phenotypes represent two or more species eggs laid in matings between different parent phenotypes should not hatch or there should be a negative correlation between hatching success and parent phenotypic difference. This is not a definitive test if there is an identical spread of colour variants among two closely related species. However, under such circumstances there should occur, by chance, several matings with extremely low or no hatching success of the eggs.

Spiderlings hatched from the egg sacs in all matings (Table 1). In total 342 spiderlings hatched from 585 eggs. The proportion of hatched eggs varied between 14.8% and 97.4%. I found, however, no negative correlation between hatching success and phenotypic difference between the parents (Fig. 17; $r_S = 0.263, p < 0.25$, Spearman rank correlation test). The phenotypic differences were estimated by comparisons between the parents and the reference specimens (see Material and methods). Hence, this supports the hypothesis that the phenotypes are a single species.

Discussion

The variation in dark coloration among *P. phrygianus* is considerable in all stages of the life-



Figs. 8-17: **8-13** Variation in dark coloration in (8-10) spiderlings newly emerged from the egg sac and (11-13) in juveniles after their first winter (three main phenotypes are shown for each stage); **14-16** Frequency distribution of percentage dark coloration on abdomen among subadults in a natural population. Samples taken (14) 30 Sept. 1982 (n = 62); (15) 5 April 1983 (n = 50); (16) 26 Sept. 1983 (n = 123); **17** Relation between hatching success of eggs and difference in percentage dark abdominal coloration between the parents.

cycle. The correlation found between the amount of darkish colours on the abdomen and femur I in adults indicates that no mosaic pigmentation occurs on a specimen. The samples from the natural population show that the amount of dark coloration on the spiders varies continuously. For convenience the phenotypes may be separated into two extremes, pale and melanic, and one intermediate type. However, it must be emphasized that this is an arbitrary classification, done merely to facilitate the description of the coloration of a certain individual. Analyses of the mating results revealed a genetic basis of the observed phenotypes. The genetics of the colour variation in *P. phrygianus* will be reported on in a separate paper (Gunnarsson in prep.). The inheritance of coloration in *P. phrygianus* is probably due to polygenic factors and hence the use of three phenotypes is not justified from a genetic point of view.

There are many examples of how the coloration of an animal affects its survival. This also applies to intraspecific variation. For instance, in industrial areas with atmospheric pollution cryptic melanic individuals of various moths are less exposed to bird predation than are non-cryptically coloured individuals (Kettlewell, 1973). The genetic basis of colour variation in *P. phrygianus* implies that natural selection can operate on the different phenotypes. A factor that influences the survival of spruce-living spiders is bird predation, which is known to cause at least 20-23% of winter mortality in populations in this particular study area in SW Sweden (Askenmo *et al.*, 1977; Gunnarsson, 1983). Bird predation may thus be a powerful selective force if the different phenotypes are affected unequally. Another factor that may exercise influence on an individual is the physiological consequences of its amount of dark coloration. Black specimens, e.g. melanic spiders, absorb heat better than light ones (Schmidt-Nielsen, 1979). Dark spiders might thus be able to prolong their foraging periods because of better heat absorption from solar radiation than light individuals. This may be important to *P. phrygianus* since winter-foraging is common in the population studied in southwestern Sweden (Gunnarsson unpubl.). However, this also means that melanic spiders in winter are probably subjected to an increased risk of being captured by foraging birds (Gunnarsson, 1985). Hence, the maintenance of colour variation in *P. phrygianus* may be due to different selective forces acting in a balancing way.

Parent phenotypes (♀ x ♂)	Phenotypic difference between parents (%)	Number of eggs hatched	Total number of eggs in sac	Proportion hatched (%)
p x p	10	24	58	41.4
p x i	30	27	44	61.4
p x i	30	30	43	69.8
p x i	30	38	49	77.6
p x m*	70	33	43	76.7
i x i	10	33	50	66.0
i x i	10	55	60	91.7
i x p (a)	10	16	67	23.9
(b)	10	20	32	62.5
i x m	40	37	38	97.4
m x p	50	9	61	14.8
m x m*	0	20	40	50.0

Table 1: Results from matings between different *P. phrygianus* phenotypes. Three parent phenotypes were distinguished: pale (p), intermediate (i) and melanic (m) (see Figs. 1-6). The phenotypic difference between parents is given as percentage dark coloration. One female produced two egg sacs from which spiderlings hatched (a, b). One male, marked with an asterisk, was used in two matings.

High local frequencies of melanism in arthropod populations are often correlated with environmental air pollution (see Bishop & Cook, 1980 for a review). It is unclear whether the melanism of *P. phrygianus* is related to industrial melanism. This cannot be decided until more is known about the geographical distribution of the colour variation. There are some records of melanism among other spiders, e.g. the linyphiid *Ostearius melanopygius* (O. P.-Cambridge) (Locket & Millidge, 1953; see also Bishop & Cook, 1980 for more examples).

Colour variation in spiders is perhaps best known in the polymorphic *Enoplognatha ovata* (Clerck). In the traditional view there are three distinct morphs or varieties, namely *lineata, redimita* and *ovata* (Locket & Millidge, 1953). Oxford (1976) observed intermediates between the three morphs and Hippa & Oksala (1979) suggested that the traditional morph distinction should be abandoned when the genetic basis of the polymorphism was analysed. However, results from breeding experiments performed by Oxford (1983) showed that a one-locus mechanism appears to control the colour variation in *E. ovata*. Recently Hippa & Oksala (1982, 1983) described a number of species closely related to *E. ovata* and this might have confused earlier results on the occurrence of the different morphs in natural populations of *E. ovata*.

Some variation in dark coloration in *P. phrygianus* was observed by Ashmole *et al.* (1978), but the number of specimens investigated was low and no melanic individuals were reported. The species *P. palilis* (L. Koch) has been suggested to be a melanic variety of *P. phrygianus* (Simon, 1929) and only a few records of *P. palilis* have been published (Bonnet, 1958). Later Wiehle (1956) and Prószyński & Staręga (1971) considered *P. palilis* and *P. phrygianus* as the same species. The continuous variation in the distribution of dark coloration, the results from the mating experiments, and the sympatric occurrence of the different phenotypes in the natural population, give credence to the assumption that *P. palilis* is a melanic variety of *P. phrygianus*. There are, however, still some unclear points about *P. palilis* and *P. phrygianus*. Koch (1870) mentions differences in spine numbers on femur IV between *palilis* and *phrygianus*. I have not observed these differences among my spiders. Koch (1870) mentions differences in spine numbers *palilis* on a limited number of spiders (females only). It is thus possible that Koch examined only a few specimens and that their lack of spines might have been due to damage.

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References

ASHMOLE, N. P., LOCKET, G. H., LODHI, A. Q. K., SMITH, C. J. & SUDD, J. H. 1978: *Pityohyphantes phrygianus* (C. L. Koch), a possible recent colonist of Britain (Araneae: Linyphiidae). *Bull.Br.arachnol.Soc.* **4**(6): 279-284.

ASKENMO, C., VON BROMSSEN, A., EKMAN, J. & JANSSON, C. 1977: Impact of some wintering birds on spider abundance in spruce. *Oikos* **28**: 90-94.

BISHOP, J. A. & COOK, L. M. 1980: Industrial melanism and the urban environment. *Adv.ecol.Res.* **11**: 373-404.

BONNET, P. 1958: *Bibliographia Araneorum* **2**(4, N-S): 3027-4230. Toulouse, Douladoure.

GUNNARSSON, B. 1983: Winter mortality of spruce-living spiders: effect of spider interactions and bird predation. *Oikos* **40**: 226-233.

GUNNARSSON, B. 1985: Interspecific predation as a mortality factor among overwintering spiders. *Oecologia (Berl.)* (in press).

HIPPA, H. & OKSALA, I. 1979: Colour polymorphism of *Enoplognatha ovata* (Clerck) (Araneae, Theridiidae) in western Europe. *Hereditas* **90**: 203-212.

HIPPA, H. & OKSALA, I. 1982: Definition and revision of the *Enoplognatha ovata* (Clerck) group (Araneae: Theridiidae). *Entomol.Scand.* **13**: 213-222.

HIPPA, H. & OKSALA, I. 1983: Cladogenesis of the *Enoplognatha ovata* group (Araneae, Theridiidae), with description of a new Mediterranean species. *Ann.Ent.Fenn.* **49**: 71-74.

KETTLEWELL, H. B. D. 1973: *The evolution of melanism.* Oxford, Clarendon Press.

KOCH, L. 1870: Beiträge zur Kenntnis der Arachnidenfauna Galiziens. *Roczn.Ces.Król.nauk.Kraków* **41**: 1-58.

LOCKET, G. H. & MILLIDGE, A. F. 1953: *British Spiders* **2**: 1-449. London, Ray Society.

OXFORD, G. S. 1976: The colour polymorphism in *Enoplognatha ovatum* (Clerck) (Araneae: Theridiidae) – temporal stability and spatial variability. *Heredity, Lond.* **36**(3): 369-381.

OXFORD, G. S. 1983: Genetics of colour and its regulation during development in the spider *Enoplognatha ovata* (Clerck) (Araneae: Theridiidae). *Heredity, Lond.* **51**(3): 621-634.

PALMGREN, P. 1972: Studies on the spider populations of the surroundings of the Tvärminne Zoological Station, Finland. *Commentat.biol.* **52**: 1-133.

PRÓSZYŃSKI, J. & STARĘGA, W. 1971: Pajaki (Aranei). *Kat.Fauny polski* **33**: 1-382.

SCHMIDT-NIELSEN, K. 1979: *Animal physiology: Adaptation and environment* (2nd ed.). Cambridge, Cambridge University Press.

SIMON, E. 1929: *Les Arachnides de France* **6**(5): 533-772. Paris.

WIEHLE, H. 1956: Spinnentiere oder Arachnoidea (Araneae). X. 28. Familie Linyphiidae – Baldachinspinnen. *Tierwelt Dtl.* **44**: 1-337.