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Determining the time required to ensure mating in bivoltine silkworm (*Bombyx mori* L.)

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Abstract. The silkworm is an economically important insect known for its unique silk production. Oviposition is an important aspect of insect reproduction which needs in-depth study. The aim of this study was to determine the effect of the mating period on the fecundity, egg fertility, and performance of bivoltine silkworm (*Bombyx mori* L). Adult males and females were allowed to mate for 1, 2, 3, 4, 5, and 6 hours respectively. The eggs laid by the mother moth were acid treated, incubated, and subsequent hatched larvae were reared following standard protocols. The treatments were replicated five times and placed in a completely randomized design. Data were collected on female fecundity, egg fertility, larval and pupal weights, larval survival, and cocoon parameters. Data were subjected to analysis of variance and means were separated using DMRT (P <0.05). The results showed that the number of eggs laid, larval weight, number of cocoons harvested, shell ratio, and effective rate of rearing were significantly higher in progeny whose parents were allowed to mate for one hour. The cocoon weight, pupal weight, and shell weight were highest in progeny whose parents were allowed to mate for five hours, but not significantly different from the ones of other mating durations. There was a negative linear relationship between mating duration and hatchability (r=-0.88) at P<0.05. The study conclusively established that mating duration of one hour in bivoltine silkworms is enough to produce the best larval and cocoon performance. Hence, one-hour mating duration can be recommended for silkworm producers in the sericulture industry.

Keywords: Fecundity, oviposition, mating duration, sericulture, progeny

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Introduction

Optimizing resource utilization is a crucial aspect of any enterprise for achieving productive efficiency. Silkworms (*Bombyx mori* L.) are a primary resource in the sericulture industry and their effective rearing is an important process for efficiency and economics. The ability to manage and manipulate silkworm reproduction is paramount for maximizing the benefits obtainable from the insect. Following emergence, both male and female silkworms are coupled for a period of six to eight hours before decoupling either naturally or artificially. Insect copulation and oviposition is a complex process driven by various physiological and behavioural factors. The physiological process involves the interplay of sensory cues, hormonal changes, and genetic mechanisms that orchestrate the reproductive behaviour of insects (Hoshino & Niwa, 2021). The male mates for prolonged duration and produces large ejaculate containing nutrients that are used by the female for reproduction and somatic maintenance (Eberhard, 1996).

In the sericulture industry, availability of high-quality eggs is an important factor for a successful silkworm rearing. In other words, good quality egg production implies characteristics and conditions that contribute to overall health, productivity, and desirable traits of silkworm. These characteristics include egg viability, egg laying capacity, uniform hatching, and good rearing performance of the progeny (Saikia *et al.*, 2023). Silkworm adults have a relatively short lifespan (3-6 days), and their reproductive capacity is often underutilized. However, the optimum reproductive potential of adult males can be achieved through multiple mating. This strategy enables a single adult male to facilitate egg laying in multiple females, thereby optimizing reproductive output.

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Determining the optimal mating duration for silkworm fertility is crucial for maximizing silk production, ensuring a consistent and abundant supply of silkworms. This knowledge helps streamline breeding programs, reducing the time and resources required for effective reproduction. By optimizing mating times, breeders can ensure healthier and more viable offspring and enhancing the genetic quality of the silkworm population. Additionally, higher fertility rates lead to increased silk yield, boosting the economic viability within sericulture. The primary focus of this study was to investigate the effect of mating duration on the fecundity, egg fertility, and performance of bivoltine silkworm.

Materials and methods

These investigations were conducted at the Sericulture Laboratory, Department of Forest Conservation and Protection, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Disease free silkworm eggs were obtained from the Ondo State Agri-Business Empowerment Center (OSAEC), Akure, Ondo State, Nigeria, while leaves from the mulberry plant (*Morus alba* L.) used in the feeding of the silkworm larvae were collected from the FRIN arboretum.

Before rearing, the Sericulture Laboratory and equipment were cleaned with teepol detergent and disinfected with 3% formalin. The sourced egg cards with the eggs were kept and protected inside newspapers. They were incubated inside silkworm rearing tray for eight days at room temperature of 25 °C, relative humidity 85% and light 16 hours and 8 hours of darkness. To ensure uniform hatching of the eggs in the same day, the eggs that had reached pinhead stage (the early developmental phase of the silkworm eggs just before it hatches) were kept under total darkness by wrapping them in black box for two days. They were exposed to light between 7:00 and 8:00 a.m. on the tenth day of hatching (Singh & Murali, 2022). Upon hatching, the newly emerged larvae were gently transferred into rearing trays. Mulberry leaves were cut into approximately 1cm × 1cm size and fed to the newly emerged larvae. The leaf size was gradually increased with the age of the insects until they could feed on whole leaves. Feeding of the larvae and cleaning of the rearing trays continued until the larvae reached the 5th larval stage.

The 5th instar larvae were mounted on plastic montage to form pupa encased in a cocoon. The cocoons were harvested and placed inside Petri dishes until adult emergence. Adult males and females were allowed to mate for durations of 1, 2, 3, 4, 5, and 6 hours in five replicates. The treatments were arranged in a Completely Randomized Design. At the end of each mating duration, the mating insects were separated and the females were placed on filter paper with cellule for oviposition. Data on the number of eggs laid by the mated female adult moth were counted and recorded.

Subsequently, the eggs underwent acid treatment for 1 hour using concentrated HCL prepared at specific gravity of 1.095 and were incubated for 10 days. The number of hatched larvae from eggs laid at different mating durations were counted and recorded. The percentage egg fertility at each mating duration was calculated by dividing the total number of hatched larvae by total number of number of eggs laid and multiplied by 100. One hundred of 1st larval instars were picked from each replicate and transferred to the rearing trays for the experiment. Larvae were fed daily with mulberry leaves throughout the period of larval duration. Data were collected on fifth instar larval survival, Percentage of cocoon harvested, fifth larval instar weight cocoon weight, pupal weight, shell weight, cocoon shell ratio, and effective rate of rearing. Cocoon shell ratio and effective rate of rearing were calculated using the formulae below:

ERR= (no of larvae spinning cocoon/no of larvae brushed) × 100

SR= (shell weight/cocoon weight) × 100

Data were subjected to analysis of variance using SAS 9.1 version software package, and means were separated using Duncan New Multiple Range Test (P<0.05). Pearson correlation analysis was carried to determine the relationship between the mating duration of bivoltine silkworm and egg hatchability.

Results

The number of eggs (n=475) laid by mated female moth at 1 hour mating duration was significantly higher than the lowest number of eggs (n=289.2) recorded when females mated for three hours, but not significantly different when compared to mating durations of 2, 4, 5, and 6 hours (Table 1). The percentage egg fertility (>70%) recorded from 1-4 hours mating durations was not significantly different, but significantly higher than egg fertility at five- and six-hours mating durations. Lower percentages (<90%) of larval survival up to the fifth larval instar were recorded at the mating durations of two and three hours. These larval survivals were significantly lower than at the mating durations of 1, 4, 5, and 6 hours. The mating duration of one hour

produced the highest (>90) cocoon which was significantly higher than the number of cocoons produced at the mating durations of two to six hours.

Table 2 displays larvae hatched from eggs produced from one-hour mating duration recorded the highest weight, which was significantly higher (P<0.05) than larval weight from the rest of mating durations. The cocoon weights at the mating periods of 2 – 6 hours were not significantly different (P<0.05), although, the mating duration of one hour recorded the least cocoon weight. The mating periods of 2–5 hours produced higher weights of pupae which were not significantly different (P<0.05), but significantly higher (P<0.05) than pupal weights obtained at the mating periods of one and six hours. The results of this study showed that the cocoon weight was lowest in progeny whose parent were mated for one hour, however, the cocoon shell weights were not significantly different (P<0.05) from the ones obtained when the parent were mated at 1–6 hours mating periods.

The highest shell ratio (21.58%) was recorded in cocoons produced by progeny whose parents mated for one hour but not significantly different from other treatments (Fig. 1). The highest and least effective rate of rearing (96.94% and 79.07%) were recorded in silkworm offspring whose parents were mated for one hour and two hours respectively (Fig. 2).

There was a strong linear negative relationship (r=-0.88) between mating duration and hatchability (P<0.05). The hatchability reduced by 88% with mating duration particularly where the mother moths were mated for two hours or more (Fig. 3).

Table 1. Effect of mating period manipulation on bivoltine silkworm egg, 5th instar larvae and cocoon parameters.

Treatment	No of eggs laid	% egg hatchability	Larval survival (%) up to the 5th instar	No of cocoon harvested
1 hour	475+70.92a	83.45+12.69a	96.8+1.71a	93.8+1.28a
2 hours	421.2+39.83ab	97.62+0.78a	88.6+0.51c	70+5.43c
3 hours	289.2+72.45b	87.43+4.88a	81.2+0.58d	72+2.0cd
4 hours	380.4+71.19ab	76.16+9.32ab	91.8+1.53bc	86.2+0.97ab
5 hours	408.8+20.09ab	52.02+16.58bc	94.6+1.32ab	79.8+2.84bc
6 hours	433.4+19.39ab	44.50+11.75c	92.4+1.54bc	77.6+1.08cd

Means with the same letter in each column are not significantly different

Table 2. Effect of mating period manipulation on bivoltine silkworm larval, cocoon and pupal parameters

Treatment	Larval weight (g)	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)
1 hour	2.49+0.11a	1.08+0.04b	0.85+0.03c	0.23+0.02a
2 hours	1.32+0.04d	1.21+0.03a	0.99+0.04a	0.22+0.01a
3 hours	2.17+0.09bc	1.18+0.05ab	0.94+0.04ab	0.24+0.01a
4 hours	2.35+0.08abc	1.17+0.02ab	0.94+0.02ab	0.22+0.04a
5 hours	2.10+0.11c	1.23+0.03a	0.99+0.02a	0.24+0.05a
6 hours	2.41+0.08ab	1.13+0.05ab	0.89+0.03bc	0.24+0.05a

Means with the same letter in each column are not significantly different

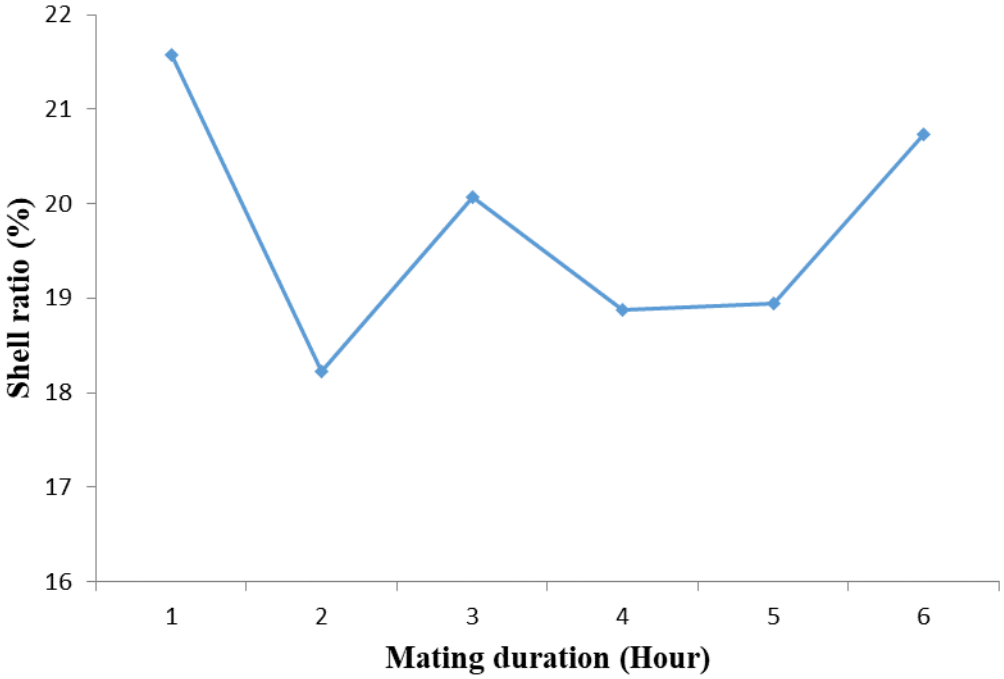

Fig. 1. Effect of mating period on bivoltine silkworm cocoon shell ratio

Discussion

In silkworm reproduction, mating is one of the decisive factors which do not only determine the total number of eggs laid but also the inducement of the regular oviposition. From this study, the highest number of eggs laid were from adult females that mated for one hour. This is in agreement with reports of Batham & Yadav (2015) that 60 minutes of coupling is enough for maximum egg laying in the silk moth. Within one hour mating duration, sufficient male secretion has been passed to the female which subsequently induces normal oviposition.

The higher number of eggs, larval survival, and cocoon production observed after a one-hour mating duration may indicate an optimal physiological timeframe for successful mating and reproductive outcomes in the silkworm. Longer mating durations (two and three hours) leading to lower larval survival might be attributed to increased energetic costs associated with prolonged mating. This could result in reduced resources available for egg production and offspring viability. The physiological mechanisms governing egg production, fertility, and larval survival may be regulated by hormonal changes during mating (Gujar & Palli, 2016). Hormonal signaling pathways could be optimized for a one-hour mating duration, ensuring efficient resource allocation for reproduction. The decline in egg fertility and larval survival beyond four hours may suggest that female moths allocate their reproductive investment strategically. Extended mating durations might lead to diminishing returns, possibly due to resource depletion or physiological stress. The highest cocoon production after a one-hour mating duration could be linked to the synchronization of physiological processes involved in silk production and cocoon formation, which may peak during the early stages of mating. The observed differences across mating durations suggest potential trade-offs in reproductive strategies. Longer mating durations may offer certain benefits, but the costs, as seen in reduced larval survival and overall reproductive success, need to be carefully balanced.

The result however negates earlier report that *B. mori* requires optimum mating duration of three hour to achieve higher fecundity (Raju, 1996). The reason for this deviation is because of differences in weather conditions, nutrition, and genotype of the silkworm strain. Our study revealed that the seminal fluid and sperm that produce more than 80% fertility and lay optimum number of eggs may have been provided by males during the first ejaculation and the second one may not be necessary, thereby saving a lot of time for the silkworm seed grainier. This will subsequently engender timely supply of adequate quality and quantity of disease-free silkworm eggs to sericulture farmers for successful harvest of cocoon crops.

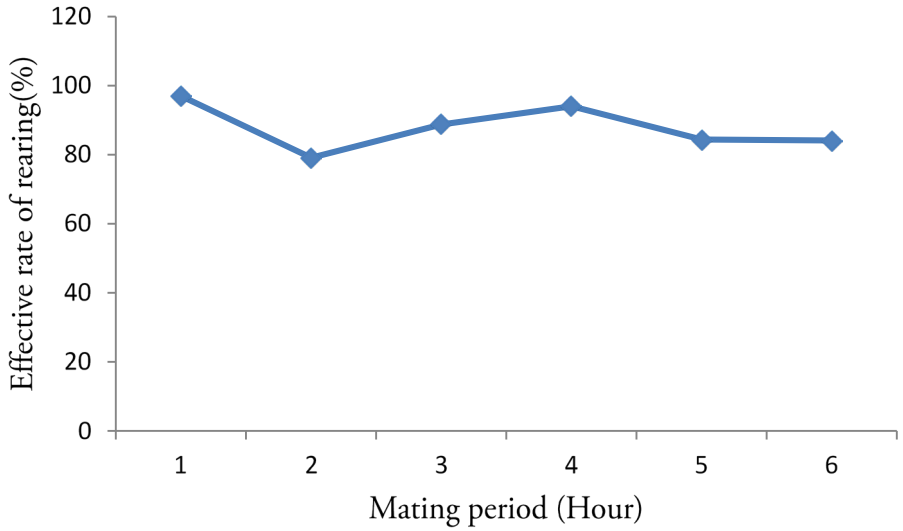

Fig. 2. Effect of mating period manipulation on insect effective rate of rearing.

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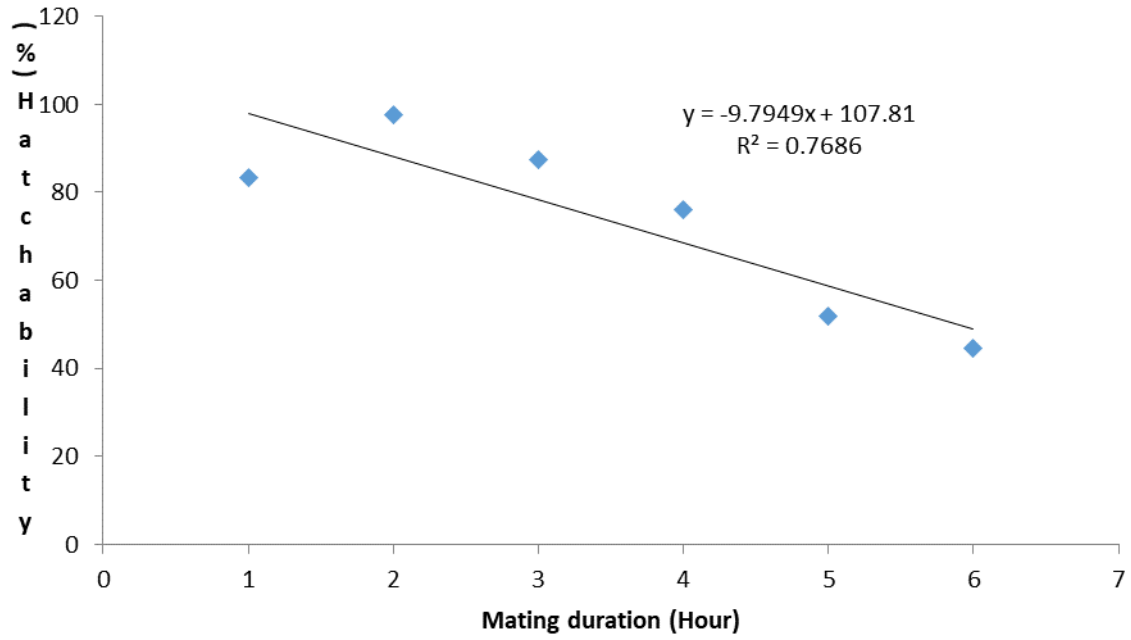

Fig. 3. Relationship between mating duration and hatchability in bivoltine silkworm.

Our investigations showed that although the highest number of eggs that hatched were from adult females mated for two hours, this was not significantly different from the ones obtained from adult females mated for

only one hour. This is contrary to an earlier report that four hours of mating duration is required for a high hatching percentage (Joan *et al.*, 2022). The hatchability of the eggs in this study declined with increasing mating duration of more than three hours for the mother moth. This is contrary to earlier reports from Jadav & Geared, (1978) and Punitham *et al.* (1978) that an increase in mating duration of *B. mori* from 3-9 hours enhanced the hatching percentage of eggs.

The higher weight of larvae from one-hour mating duration suggests that this specific mating duration positively influences larval development. This could be attributed to genetic or environmental factors influenced by the shorter mating period, impacting the initial growth stages of the silkworm. The variations in cocoon weights and pupal weights across different mating durations indicate that physiological processes during pupation and cocoon formation are influenced by the duration of parental mating. This may be related to nutrient allocation and utilization during these critical developmental stages. The differences in shell ratios among mating durations highlight potential variations in silk quality. The shell ratio is an important parameter in silk production, influencing the characteristics of the final silk product. The higher shell ratio in the one-hour mating duration suggests a potential for improved silk quality.

In silkworm rearing, effective rate of rearing (ERR) is an important parameter for assessing silkworm rearing performance (Radhakrishnan & Perisamy, 1986). In this study ERR was highest in the progeny whose parents were mated for just one hour. As stated by Saikia *et al.*, 2023, good quality of egg refers to richness of egg laying, viability, uniform hatching, and subsequently good rearing performance of this progeny. All of these parameters were obtained from the mother moth mated for period of one hour.

Conclusion

Our studies showed that silkworm performance parameters, such as the number of eggs laid, larval survival up to the 5th instar, number of cocoons harvested, larval weight, shell ratio, and effective rate of rearing were best obtained where the mother moths were mated for just one hour. Percent hatchability was highest in progeny whose parents were mated for two hours but it was not significantly different from others, except from the ones whose parents were mated for five and six hours. Though other parameters such as, cocoon weight and pupal weight were highest in progeny produced by moth mated for five hours, however, the performance in these capacities were not significantly different from the ones obtained in progenies of others. The shell weight too was highest in progeny whose parent were allowed to mate for five hours but not significantly different from the ones whose parent were mated for one hour. Since the best performance can be realized when bivoltine silkworm are mated for one hour without any decadence on the insect's economic parameters, it will be best at egg production stage to allow the insect mate for an hour thereby saving time without any loss.

Author's Contributions

AOT and AAE conceptualized and designed the study. AAE and EJA conducted the experiment. AAE analysed the statistical data and drafted the manuscript. AOT and EJA reviewed and proof read the manuscript. All authors read and approved the final manuscript.

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The present study was self-funded by the authors.

Data Availability Statement

All data supporting the findings of this study are available within the paper.

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Ethics approval

Insects were used in this study. All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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تعیین زمان لازم برای اطمینان از جفت‌گیری در کرم ابریشم دو نسله (*Bombyx mori* L.)

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چکیده

کرم ابریشم حشره‌ای مهم و اقتصادی است و علت این موضوع، تولید ابریشم منحصر به فردش می‌باشد. تخم‌گذاری یکی از جنبه‌های مهم در تولید مثل حشرات است که نیاز به مطالعه‌ی عمیق دارد. هدف از این مطالعه، بررسی تعیین تأثیر دوره جفت‌گیری بر زادآوری، باروری تخم و عملکرد کرم ابریشم دو نسله‌ی (*Bombyx mori* L.) بود. به نرها و ماده های بالغ به ترتیب به مدت ۱، ۲، ۳، ۴، ۵ و ۶ ساعت اجازه جفت‌گیری داده شد. تخم‌های گذاشته شده توسط پروانه مادر تحت درمان با اسید قرار گرفتند، انکوبه شدند و لاروهای تفریخ‌شده بعدی طبق روش‌های استاندارد پرورش داده شدند. هر تیمار پنج تکرار داشت و در قالب طرح کاملا تصادفی انجام شد. در ادامه، داده‌های باروری ماده، باروری تخم، وزن لارو و شفیره، بقای لارو و پارامترهای شفیرگی جمع‌آوری شد. داده‌ها به روش واریانس مورد تجزیه و تحلیل قرار گرفتند و میانگین‌ها با استفاده از DMRT تفکیک شدند ($P < ۰/۰۵$). نتایج نشان داد که تعداد تخم، وزن لارو، تعداد پیله‌های برداشت‌شده، نسبت پوسته و سرعت مؤثر پرورش در نتاجی که والدین آنها اجازه جفت‌گیری به مدت یک ساعت را داشتند، به‌طور معنی‌داری بیشتر بود. وزن پیله، وزن شفیره و وزن پوسته در نتاجی که والدین آنها مجاز به جفت‌گیری به مدت پنج ساعت بودند بالاتر بود، اما تفاوت معنی‌داری با سایر دوره‌های جفت‌گیری نداشت. بین مدت زمان جفت‌گیری و تخم‌گذاری ($r=۰/۸۸-$)، رابطه خطی منفی ($P<۰.۰۵$) وجود داشت. این مطالعه به طور قطع نشان داد که فراهم نمودن یک ساعت زمان جفت‌گیری در کرم‌های ابریشم دو نسله، مهمترین نقش را در افزایش عملکرد لارو و شفیره داشت. از این رو، مدت جفت‌گیری یک ساعته را می‌توان برای تولیدکنندگان کرم ابریشم در صنعت نوغانداری توصیه کرد.

کلمات کلیدی: باروری، تخم‌گذاری، مدت زمان جفت‌گیری، نوغانداری، نتاج

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