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Half male half female: A rare prodigy of Gynandromorphism in Tasar silkworm *Antheraea mylitta* D (Saturniidae: Lepidoptera) with special reference to its procreative behaviour

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Abstract
Gynandromorphs are organisms which exhibit both male and female characteristics. This does not necessarily mean that it possesses both male and female reproductive organs, such as seen in hermaphroditic animals, as this article highlights about half- male, half female moth of *Antheraea mylitta* Drury spotted in Central Tasar Research and Training Institute, Ranchi, Jharkhand still expressed a female gynandromorphy in behaviour and morphologically appears to be known as a bilateral gynandromorph. This particular study has proved that genitalia of gynandromorph was similar to the female reproductive system and thus it could able to mate with normal male and that coupling has resulted in production of fertile offspring. A total of 133 eggs were laid by gynandromorph moth and 77.44% of hatching has been obtained. Longevity study showed that gynandromorph moth survived up-to 10 days. Further, the study on gynandromorphs offspring revealed that they showed normal growth and development in their life cycle and their economic characteristics has been documented.

Keywords: gynandromorph, half- male, half female, *Antheraea mylitta*, procreative

1. Introduction
Gynandromorphism is a rare phenomenon in both natural and laboratory environments [21]. Gynandromorphs are creatures that are part female (gyn) and part male (andro). Gynandromorphy is thought to occur when female egg cells develop with two nuclei- so that one nucleus contains a single Z chromosome and the other contains a single W. When that egg is fertilized by sperm carrying two male Z chromosomes, the egg develops with both ZZ (male) and ZW (female) chromosomes. The organism then develops with half of its body containing male ZZ cells while the other half contains female ZW cells. If this chromosomal mix-up occurs early on in the animal's development, before many of their cells begin to divide, it can result in the sort of perfect bilateral split. In addition, many individuals can develop as mosaic gynandromorphs, with only some portions of their bodies developing as the opposite sex. The study of both bilateral and mosaic individuals offers potential insights into the range of phenotypes that may be produced by developmental plasticity in genitalic development because individuals experience unusual genetic and hormonal environments (both male and female) at the genitalic midline compared with normal individuals. It is worth noting that hormones have only recently been thought to be important in insect sex-determination; however, gynandromorphs are considered as one of the best evidence for cell-autonomous (i.e., genetically predetermined) sex determination [1]. The factors like temperature, ultraviolet light, viral infections, mutations, nuclear power plant disaster and interspecific hybrid crosses are associated with the development of such irregularities [22, 17]. Gynandromorphism has been reported from 69 families of insects, across 13 orders [7]. In Lepidoptera, it is described in moths of the families *viz*., Saturniidae, Noctuidae, Geometridae and in butterfly families *viz*., Nymphalidae, Papilionidae etc. [9, 18, 12, 20, 10, 2, 25, 13, 8].
Tropical tasar silkworm, *Antheraea mylitta* Drury, is a polyphagous non-mulberry silkworm used for production of commercial silk and distributed in diversified ecological and geographical regions ranging from 12°N to 31°N lat. and 72°E to 96°E long. About 44 eco-races have been reported in tasar silkworm [24] and mainly feeds on *Terminalia arjuna* (Arjun), *Terminalia tomentosa* (Asan) and *Shorea robusta* (Sal) which are primary food plants species.

Tasar silkworm is semi-domesticated and produces 2-3 crops in a year (July-August, September-October and November-February). The occurrence of gynandromorphs is very rare in both wild and commercial populations of the tropical tasar silkmoth, A*ntheraea mylitta* D. Earlier, the gynandromophy in tasar silkmoth, *Antheraea mylitta* Drury had been described from India [23, 8], but in this manuscript behaviour of Gynandromorphs with special reference to its reproductive ability to produce fertile eggs has been reported for the first time.

2. Materials and Methods

The experiment was conducted at the Central Tasar Research and Training Institute, Ranchi.
Ecorace: Daba
Crop: Trivoltine

2.1. Mating and oviposition behavioural study of gynandromorph moth

The gynandromorph moth was released into a group containing of normal male and female moths in grainage to study its natural mating instinct. Once after the gynandromorph moth coupled with male/female, it is decoupled after 8 hours of mating duration. Decoupled gynandromorph moth was transferred to oviposition device and eggs were collected at regular intervals till cessation of egg laying.

2.2. Egg washing and Incubation care

The collected eggs were washed using 5% Depuratex for the surface cleaning and sterilization of tasar silkworm eggs. After washing, the eggs were shade dried and incubated eggs at 28 ^{0}C temperature and 70-80% R.H for proper embryonic development.

2.3. Disinfection of rearing site and plantation

Before commencement of rearing, 2% beaching powder solution is sprayed on the plants and ground. During rearing, bleaching powder is sprinkled regularly at an interval of 4 to 5 days to maintain hygienic condition in the rearing site.

2.4. Rearing of gynandromorphous tasar silkworm, A*ntheraea mylitta* D

Type of rearing: Outdoor rearing
Food plant: *Terminalia arjuna*
Nutrient dosage: FYM-3kg/plant & NPK @100:50:50 kg/ha/yr
Season of rearing: August-September
The larvae hatched out from the eggs of gynandromorph moth are brushed on the arjun plantation which are pruned at 3' ft. height and maintained in 4' x 4' spacing for chawki rearing at CTR&TI, Ranchi. After chawki rearing of young age silkworm, the rearing from III instar onwards is conducted on 4 years and above economic plantations of *Terminalia arjuna*. Cocoons are harvested 6-7 days after spinning when the cocoons become hard. The portion of the twig near the ring is cut to make the cocoon free from branches.

2.5. Evaluation of economic characteristics

a. Larval weight (g): Larva is weighed on daily basis from hatching till spinning using digital balance with 0.001g sensitivity.

b. Cocoon weight (g): Weight of cocoons is recorded using digital balance with 0.001g sensitivity after six to seven days of complete spinning.

c. Pupal weight (g)
After recording cocoon weight, the cocoons were cut open and pupae are weighed using digital balance with 0.001g sensitivity.

d. Shell weight (g)
After recording pupal weight, the empty shells are weighed using digital balance with 0.001g sensitivity.

e. Shell ratio (%)

$$\text{Shell ratio (\%)} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

f. Statistical analysis
The data were analyzed using SPSS statistical package.

3. Results and Discussion

The two-coloured silk moth of semi-domesticated DABA trivoltine race spotted in CTR&TI, Ranchi, Jharkhand appears to be known as a bilateral gynandromorph, where the male-female characteristics are split almost directly down the middle. Male and female characters were observed both on dorsal and ventral surface of the body from the half of the abdomen towards right side along with wings bearing brown colour and half of the abdomen towards left side along with wings bearing grey colour respectively (Plate 1). The colour of the leg portion also indicated half-male and half-female with brown and grey colours respectively. The size of the legs is bigger in female part compared to male part. The results are in agreement with Hessel [14] and Bridgehouse [4] described Bilateral gynandromorphy in *Automerisio* Fabricius and *Hyalophora cercopia* (Lepidoptera: Saturniidae).
This bilateral gynandromorphy moth possesses bipectinate antennae which are broad in male side and narrow in female side. The body length measures about 3.8 cm. The size of both forewing and hind wing, as well as the eyespots on the respective wings, are more prominent and broad in the female part than male part. The length and mid width expanse of forewing and hind wing of male side is 55.5 & 30.0 and 38.5 & 27.8 mm respectively. Similarly, the length and mid width expanse of forewing and hind wing of female side is 65.0 & 34.0 and 45.5 & 32.0 mm respectively. Forewing is sub-triangular in both the male and female side. But, in female forewing, subcoastal region is not curved, apical margin not extended, apical angle is pointed and termen is almost straight. Whereas in male forewing, subcoastal region is curved, apical margin is extended forward and curved, apical angle is prominently curved and termen appears to be S-shaped. The present type was similar to schizophrenic kind, in which male and female behaviors expressed concurrently in their respective body parts [19]. The gynandromorph moth size is comparatively smaller when compared to normal male and female size. The dorsal view of normal male and female along with gynandromorphy moth is depicted in Plate 1. Similarly, Campos *et al.* [5] reported gynadromorphism in urban ant *Monomorium floricola* (Jerdon) in laboratory conditions with nine different combinations of male and female tissues and their development from egg to adult was around 74.6 days. Many possible genetic mechanisms of gynandromorphism

have been proposed, including loss of a sex chromosome during mitosis, genetic modification by endosymbionts, and double fertilization of binucleate eggs [16]. For Lepidoptera, and presumably for the *Lycaeides* bilateral gynandromorphs, double fertilization of a binucleate egg is expected to be the most common mechanism of gynandromorphism [6, 3]. The reports of Eric Werner [11] suggest that the root cause of gynandromorphism is internetwork links between allelic developmental control networks responsible for sex differences lying on allelic chromosomes. Each parent contributes a distinct set of allelic chromosomes that contain an allelic but different developmental network for an organism. The mixture of these networks leads to the ontogeny of the organism. When subnetworks responsible for the sexual dimorphism of a species are interlinked in the same developing individual organism, then that organism can exhibit aspects of both sexes. The linkage causes a jump from a developmental parental network of one sex to the opposite sex developmental parental network. This link leads from one network responsible for the morphology of one sex to the activation of the opposite sex morphology and function. The more linkages there are between opposite sex developmental networks the more variable the gynandromorph phenotype.

The question arises whether this gynandromorphous moth could able to mate with both male and female? And if copulation occurs, would the offspring produced be fertile or sterile? This article highlights about half- male, half female moth still express a female gynandromorphy. Interestingly, the copulation is observed between gynandromorph female with a normal male and the mated gynandromorph female could able to reproduce fertile eggs. The egg rhythmicity of gynandromorph female on different days is observed. The total eggs laid and its hatching percentage is represented in Table 1. Around 110 eggs are laid upto three days, 16 eggs on fourth day, 6 eggs on fifth day and 1 egg is laid on sixth day, out of which 88 eggs got hatched into larvae from the batch of 110 eggs. Interestingly, 14 eggs got hatched into larvae out of the 16 eggs laid on fourth day and 1 egg got hatched into larvae out of the 6 eggs laid on fifth day**.** The overall hatching percentage observed to be 77.44%. The hatched out larvae are reared in the fields of CTR&TI, Ranchi upto cocoon formation (Plate 2). The growth of larva in terms of larval weight from first day of hatching to till the start of spinning is plotted in Figure 1. The growth rate was slow upto 16 days and thereafter the growth started to increase at a faster rate double the initial weight. The initial hatched out larval mean weight was approximately weighing 0.008g.

The fifth instar mean larval weights were recorded during the same rearing season for both gynandromorphous and normal female moth laid batches. The appearance, growth and development of gynandromorphous offsprings were similar to the normal female moth laid ones. A maximum mean larval weight of 30.204g is noticed in the worms of gynandromorphous moth laid ones, but however the greater value was recorded in normal female layings when compared to the gynandromorphous layings (36.00g). The total larval life cycle lasted upto 35 days. After spinning or cocoon formation, the cocoons were harvested and the economic parameters like fresh weight of cocoon, shell and pupa were examined. The mean cocoon, shell and pupal weights of gynandromorphy and normal female moth's offsprings were observed to be (9.648g, 1.652g & 7.990g) and (12.753g, 2.106g & 10.646g) respectively. The maximum shell percentage of 17.120% has been recorded from gynandromorphy moth's offsprings and normal female layings recorded 16.513% (Table 2). Even though the values for cocoon, shell and pupal weights recorded in gynandromorphous were low but it has contributed to the tasar cocoon production.

The gynandromorphous moth used for longevity studies showed survivability upto 10 days. Dissection of gynandromorphic moth revealed that the genitalia is similar to the female reproductive system and female copulatory apparatus is present (Plate 7). Hence the normal male when mated with these gynandromorphic moths could able to reproduce and lay fertilized eggs but the fecundity of gynandromorphic female moth (133) was less when compared to normal female (200-250). As reported by Chaudhuri *et al.* [8], virgin normal female tasar moth, when mated with predominant male gynandromorphs laid fertile eggs. A similar reproductive behavior has been reported in gynandromorphy *Drosophila melanogaster* by Napolitano and Tompkins [15]. It is also observed that the fat content in the gynandromorph moth is more on male side compared to female side.

Table 1: Egg rhythmicity of tasar gynandromorphous moth, *Antheraea mylitta* Drury

No. of oviposition days	No. of eggs laid	Hatched out worms	Individual days Hatching %
Upto 3 days	110	88	80
4th day	16	14	87.5
5th day	6	1	16.67
6th day	1	0	0.00
Total no. of eggs laid		133	
Total no. of worms hatched out		103	
Overall hatching %		77.44%	

Table 2: Economic characteristics of tasar silkworm produced by gynandromorphous moth, *Antheraea mylitta* Drury

Parameters	Gynandromorphous moth layings	Normal female moth layings
Max. Larval weight (g)	30.204±0.701	36.000±1.027
Cocoon weight (g)	9.648±0.598	12.753±0.744
Shell weight (g)	1.652±0.168	2.106±0.143
Pupal weight (g)	7.990±0.466	10.646±0.601
Shell ratio (%)	17.120±1.076	16.513±0.223

Values represent data mean± standard error

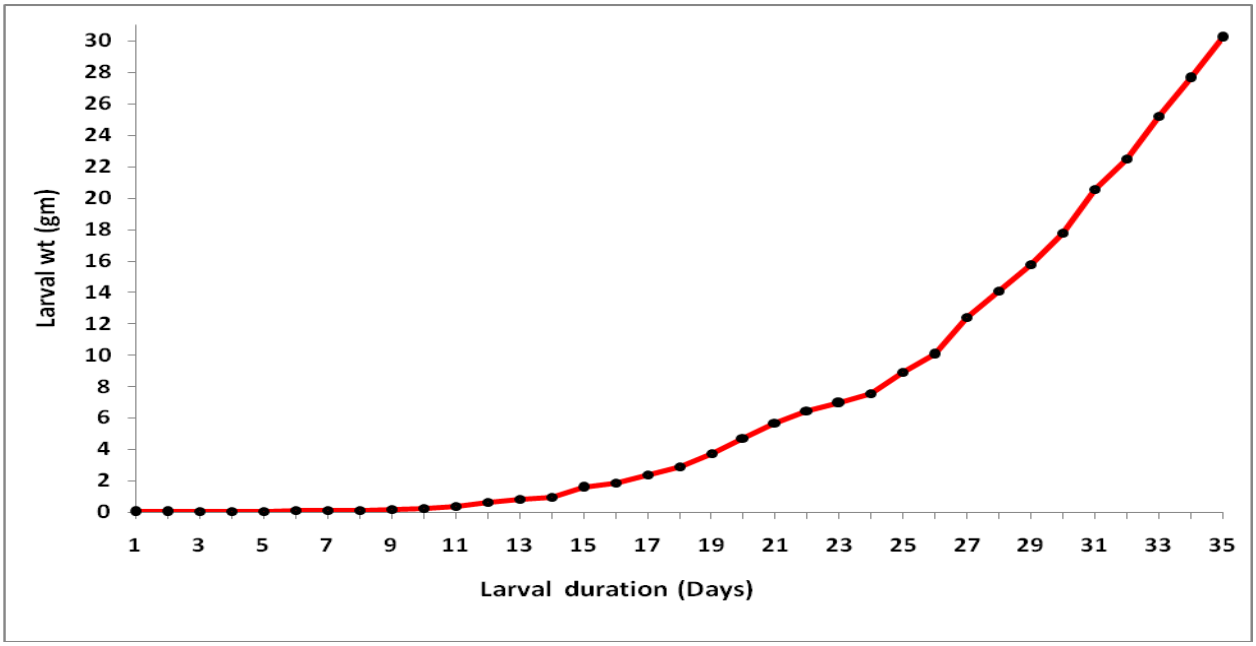

Fig 1: Growth curve of larvae produced from tasar gynandromorphous moth, *Antheraea mylitta* Drury

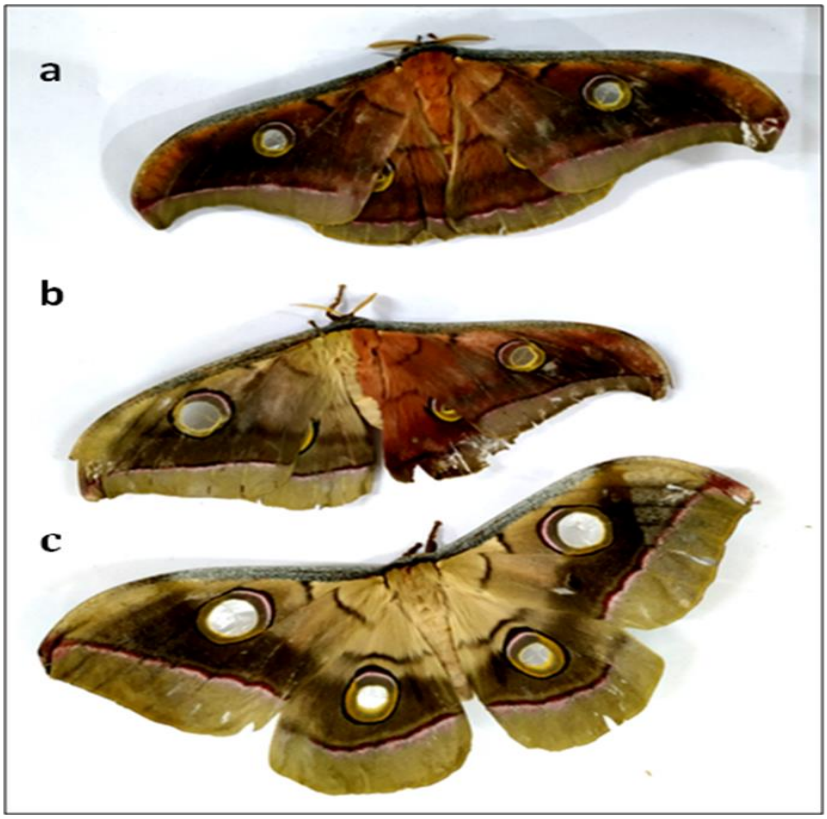

Plate 1: Comparative dorsal view of tasar silk moth, *Antheraea mylitta* Drury (Lepidoptera:Saturniidae) (a) Male (Normal), (b) Gynandromoph, (c) Female (Normal)

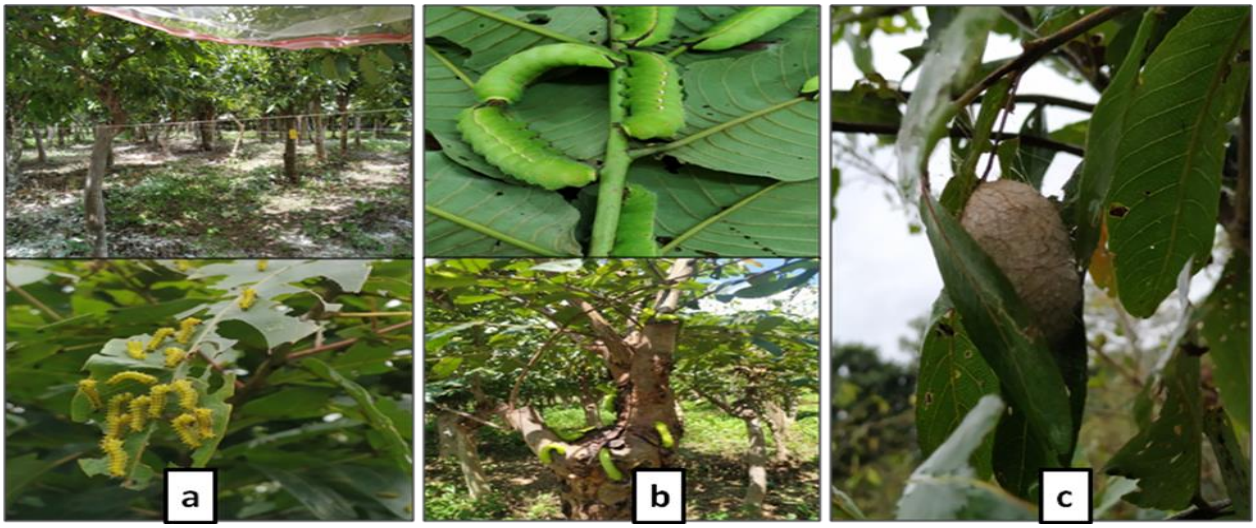

Plate 2: Rearing of hatched out worms from gynandromorphous moth laid eggs under nylon net at CTR&TI, Ranchi (a) Chawki rearing (b) Late age rearing (c) Cocoon formation

4. Conclusion

Half-male, half-female moth still expressed a female gynandromorphy. The study proved that even though the gynandromorph moth possesses both male and female characteristics externally, the internal reproductive system was almost similar to that of female. Hence this particular moth could able to mate with normal male and produced fertile offspring which exhibited normal growth and development in their life cycle and economic characteristics *viz.*, larval, cocoon, shell and pupal weights. These Gynandromorphs may be useful models for the study of sex determination, modularity and evolvability. Gynandromorphism being a rare phenomenon and gynandromorphic mutations help reveal the developmental processes that facilitate the evolution of new phenotypes in insects.

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