A difference in pupal morphology between the sibling species *Leptidea sinapis* and *L. reali* (Pieridae)

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Abstract. The sister species *Leptidea sinapis* and *L. reali* are virtually identical and species identification has so far only been possible through DNA-sequencing or genital preparation. Here, I show an interspecific difference in pupal morphology. The pupal antennae of *L. sinapis* are white with a well-defined longitudinal pink line in the middle, while those of *L. reali* are predominantly pink with only scattered white pigments close to the margin on both sides.

Introduction

In 1988, it was discovered that the formerly well-known and well-studied butterfly species *Leptidea sinapis* actually consisted of two separate species: *L. sinapis* and *L. reali* (wood white/ Real's wood white) (Réal 1988; Reissinger 1989; Lorcovic´ 1993). The two species are morphologically virtually identical, and have so far only been distinguishable using either genital preparation (Lorcovic´ 1993; Mazel 2005), DNA sequencing (Martin et al. 2003, Friberg et al. 2007), or male mating behaviour (Friberg et al. unpubl. ms). Both species are present in most parts of Europe, and although they appear to be partially partitioned in habitat in most parts of their distribution they are still often found syntopically and synchronically at the same sites (Beneš et al. 2003; Vila et al. 2003; Amiet 2004; Friberg et al. 2007). The species are reproductively isolated, and the isolation is implemented by the females' ability to distinguish between con- and heterospecific males; males court both con- and heterospecific females, while females only accept to mate with conspecifics both in the field (Friberg et al. unpubl. ms.) and in the laboratory (Freese & Fiedler 2002; Friberg et al. unpubl. ms.).

So far most studies of the sister species *L. sinapis* and *L. reali* (see Martin et al. 2003) have concerned either their distribution (Mazel 2002; Beneš et al. 2003; Mazel and Eitschberger 2003; Vila et al. 2003; Amiet 2004), their habitat partitioning (Beneš et al. 2003; Vila et al. 2003; Amiet 2004; Friberg et al. 2007) or their sexual behaviour (Freese and Fiedler 2002; Friberg et al. unpubl. ms.). Only a few studies have further analyzed potential morphological differences, and then only adult differences and primarily genital differences between the species (Lorkovic' 1993; Mazel 2005). No studies have so far dealt with potential species-specific morphological differences in other developmental stages.

Here, I show an interspecific difference between *L. sinapis* and *L. reali* that allows accurate species recognition based on pupal morphology. The two species differ in coloration of the pupal antennae, with *L. sinapis* having a more distinct edge between the inner pink stripe and the surrounding white pigmentation than *L. reali*.

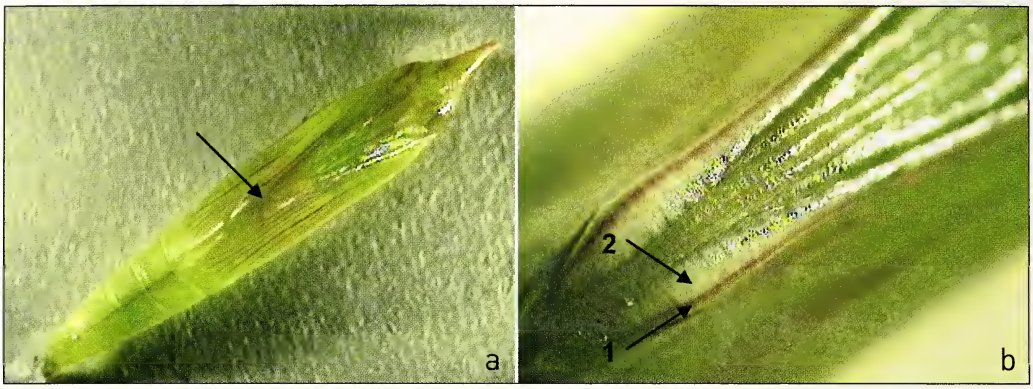

Fig. 1a. The ventral side of a *L. sinapis* pupa at 10 × magnification. The arrow indicates the pupal antennae that are closed up at 40 × magnification in figure **1b**, where arrow 1 indicates the inner well-defined central pink line that runs along the length of the antenna and arrow 2 indicates the species-specific white area that surrounds the central line.

Material and methods

Ten hibernating pupae of each species and sex (i.e. in total 40 individuals) were randomly chosen and species determined blindly; hence, the species affiliation of the pupae were not known to the identifier (MF) prior to examination. Pupae descended from parents of known species affiliation that were mated in the laboratory at the Zoological Department of Stockholm University. The laboratory population was founded in 2002 with eggs from nine wild-caught *L. sinapis* and ten *L. reali* females collected in two different populations: Riala (located 50 km north of Stockholm, 59° 30' latitude) and Kronängen (located 100 km south of Stockholm, 59° 0' latitude). The egg-laying females were species determined *post mortem* using genital preparations and DNA-sequencing (for rationale see Friberg et al. 2007). During 2005 and 2006 55 wild *L. sinapis* and 15 *L. reali* females were captured in Riala in order to maintain the genetic variation in the laboratory population. Pupae were photographed using a digital stereo magnifier (Motic 4095) at 10× magnification and at 40× magnification.

Results and discussion

All 40 examinations resulted in correct species determinations. Species determination was possible because of a difference in the antennal coloration of the pupae. The antennae of a *L. reali* pupa are coloured in pink with only scattered white pigments on both sides of the pink central line, while the antennae of a *L. sinapis* pupa are pink only in a stripe in the central part of the antennal areas, and the lateral parts are almost exclusively white with very few spots of pink (Figs 1, 2). Hence, the difference in coloration of the pupal antennae allows accurate species identification. Correspondingly, the whole *L. reali* pupa often appears darker with more red pigments than a *L. sinapis* pupa that is dominantly green, although the general pupal coloration is overlapping and the two species cannot always be distinguished only based on the pupal pigmentation. After the termination of diapause the *L. sinapis* pupae turn darker and their pupal antennae

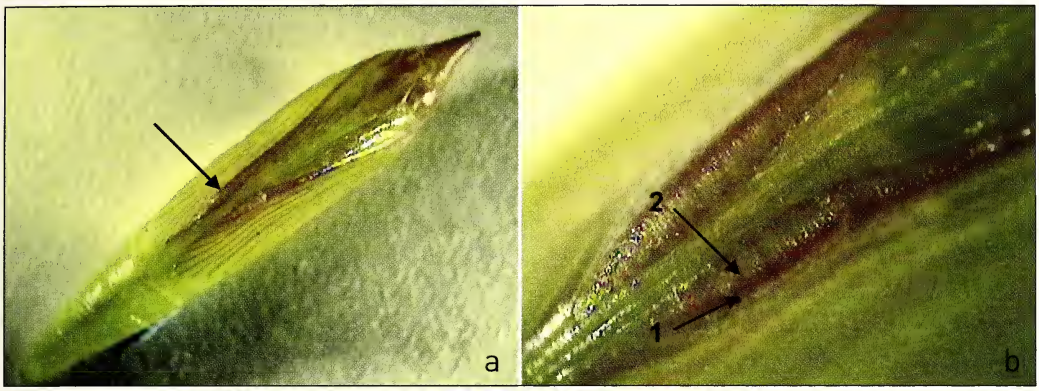

Fig. 2a. The ventral side of a *L. reali* pupa at 10 × magnification. The arrow indicates the pupal antennae that are closed up at 40 × magnification in figure **2b**, where arrow 1 indicates the undefined central pink line that runs along the length of the antenna and arrow 2 indicates the pale pink area that surrounds the central line.

become more pigmented due to the ongoing metamorphosis. *L. sinapis* pupae that are close to eclosion are therefore difficult to distinguish from those of *L. reali*.
So far most research effort concerning the biology of *L. sinapis* and *L. reali* has focused on the adult stage. This study of pupal morphology resulted in the finding of a character that makes accurate species determination possible, which highlights the importance of studying all life stages when searching for species-specific characters.

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