The Effects of *Cervus nippon* on Two Key Ecological Drivers Controlling Populations of *Plebejus argus* on Heaths: Larval Food Sources and Mutualistic Ants

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**Authors’ contributions**

Author SRW carried out all the data collection and analysis. He also wrote the first full draft of this manuscript including producing all the initial drafting of text and all the final figures. Author AD supervised this project throughout, advising on experimental design, data analysis and project write up. She conducted the final drafting of this paper. All authors read and approved the final manuscript.

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**ABSTRACT**

This study investigated the effects of grazing by invasive sika deer, *Cervus nippon* on the abundance of the silver-studded blue butterfly *Plebejus argus* on a lowland heath system in the UK. *Plebejus argus* is a rare species whose UK stronghold is lowland heath, where it is dependent on a mutualistic relationship with species of *Lasius niger* ants. *Cervus nippon* is an invasive species but genetically and ecologically closely related to native red deer *C. elaphus* and so may have positive as well as negative ecological effects. This study examines the relationship between the incidence of *C. nippon* and the abundance of *P. argus* and tests the effect of deer i) directly via their impact on vegetation structure and composition, ii) indirectly their impact on the abundance of *L. niger* ants. Data were collected from 37 plots of heathland in Dorset, measuring 50 m by 50
which were differentially grazed by deer. The abundance of *P. argus* was found to be significantly higher in areas with high incidence of *C. nippon* and the best predictor of butterfly abundance was the abundance of *L. niger* ants rather than the abundance of butterfly larval food plants. We conclude that this result provides evidence for an important indirect impact of grazing via manipulating habitat suitability for a key mutualistic species.

Keywords: Sika; grazing; Lasius ants; mutualist myrmecophile; silver-studded blue butterfly; lowland heathland.

1. INTRODUCTION

The silver-studded blue butterfly, *Plebejus argus*, is a conservation priority UK species [1,2] that is noted as vulnerable on the Red List of British butterflies using IUCN criteria [3]. It is at the northern edge of its global distribution range in the UK and is most often associated with heathland habitats although it also occurs on acid grasslands [4]. Its populations in Dorset and Hampshire, UK, are most often associated with wet or humid heath [1,5,6], but also on dry heath after a fire or cut [5]. On lowland heath elsewhere across its UK range *P. argus* is associated with dry heath, such as in Suffolk [6] and West Sussex [7], and both dry and humid heath in Devon [8,9]. *P. argus* is one of the relatively few obligate myrmecophilous lycaenids in Europe [10], whereby its populations are strongly linked to ants. The butterfly can also be described as an oligo-symbiont as only two species of ant (*Lasius niger* and *L. alienus*) are used in its lifecycle [11]. The distributions of *P. argus* life stages are all associated with the distribution of *L. niger* or *L. alienus*; on heathland *P. argus* are always found next to or very near the nests of these ants [12]. Research based on GPS tracking of *P. argus* indicates that they fly slowly in winding circles round favourable sites while flying quickly across unfavourable sites [13].

Today, much European heathland has been lost to other land uses, and effective conservation of remaining fragments requires defoliation management to prevent successional change to woodland [14]. Current management techniques to halt succession on heathland include the use of domestic grazers to graze patches or whole areas of habitat [15-17] but burning [18] and cutting [19] are also used. Large herbivores have always played an important role in maintaining open landscapes through grazing, treading, dunging and urination [20,21] and it is known that deer can exert cascading effects by indirectly modifying the composition and physical structure of habitats [22,23]. There is also the potential for wild grazers to be utilised such as rabbits *Oryctolagus cuniculus* [24] and most particularly red deer *Cervus elaphus* [25-28]. Currently there is little information on the potential positive and negative roles of invasive sika deer *C. nippon* on heathlands and on the extent to whether sika deer can create favourable habitat for other plant and animal communities.

*Cervus nippon* are native to south-east Asia and were first introduced to Britain and Ireland in the late 1800’s [29]. They are generalist feeders that can quickly change their behaviour to adapt to changes in environmental variables such as food availability [30]. As *C. nippon* become widely established they may assert an appreciable influence on an area’s ecology [31]. There is no current information on the effects of *C. nippon* on thermophilous insects such as butterflies. Therefore, the resulting information shows the potential *C. nippon* has upon heathland sites to assist in the conservation of *P. argus*. This information could also be used generally to produce more effective management schemes on sites to benefit *P. argus* populations. Understanding the effect of grazing on the population size of *P. argus* requires knowledge of its impact on two key components: i) availability of food for larval and adult *P. argus* and ii) availability of *Lasius* ants. The aim of this study was to quantify and compare the changes that may be affecting *P. argus* either by changing food plant availability and suitability for larvae or adults, with those by affecting populations of *Lasius* ants.

2. METHODS

2.1 Study Site

The study site used was Arne Nature Reserve in south-east Dorset, UK and is managed by The Royal Society for the Protection of Birds (RSPB). Included in it are approximately 608 ha of grasslands, saltmarsh, woodland and heathlands, of which 158 ha is dry heath and 43 ha is wet heath. The only large mammal grazers
on the site during the study period were *C. nippon* which during the time of this study occurred at a range of densities across the site with total population size being estimated as approximately 700 animals giving an average of more than one deer per ha which is extremely high (Royal Society for the Protection of Birds (RSPB) [32].

2.2 Sampling Design

A vegetation survey was carried out of all heathland at Arne during the summer of 2010 to establish the location of all main homogeneous areas of dry and wet heath of heathland at Arne. Areas were assigned as either ‘dry heath’ or ‘wet heath’ by using published guidelines for heath type [33], where wet heath contains *Calluna vulgaris*, *Erica tetralix*, *Sphagnum compactum* and *S. tenellum*, and dry heath contains *C. vulgaris*, *E. cinerea* and *Ulex minor*. In order to assess the impact of *C. nippon* on *P. argus*, plots measuring 50m by 50m were established in random locations within homogeneous stands of vegetation across the wet heath habitat areas. A total of 22 wet heath and 20 dry heath plots were established (the number of plots was determined by the availability of areas of suitable habitat) (Fig. 1). Five of the dry heath plots (numbers 1, 3, 4, 7 and 10) were later discarded from the analysis as no *P. argus* were recorded here in three visits made within the middle of the flight period.

The abundance of *P. argus* on each plot was assessed by employing a transect walk over the flight period based on the line transect method [34,35] but adapted into a circular transect walked at each site. Each circular transect had a radius of 20 m, with one transect per plot, and each plot being walked along the same transect route three times within the middle of the *P. argus* flight period, with a mean value taken. This method is particularly effective because of the sedentary nature of *P. argus*.

All butterfly surveys were carried out when the weather was warm, sunny, and under calm conditions. *P. argus* abundance was recorded on each site a total of 10 times every two days during the middle of the flight period, from the last week of June to mid July.

![Fig. 1. The location of dry (red) and wet (blue) survey plots at Arne nature Reserve, Dorset, UK](image-url)
The incidence of sika deer on each plot was measured using the indirect index technique of recording standing crops of faecal pellets on each quadrat in midsummer [36-38]. Pellet counts were averaged from within three randomly located 5m radius circular areas per site. All pellets present were counted.

Vegetation structure and plant community composition variables were recorded within each plot by using three randomly placed 2 m x 2 m quadrats within each plot and averaging results. The vegetation structure variables recorded were: mean vegetation height, mean percentage cover of vegetation and vegetation volume. The last of these was estimated by visually recording the percentage occupancy of slices of the plot cube at 10 cm height intervals and using these to calculate volume occupancy per slice above ground level. These were summed to give a total vegetation volume estimate for each quadrat. Plant community composition was assessed by recording the percentage cover of each vascular plant species within each quadrat and then recording the mean for the plot. The abundance of *L. niger* and *L. alienus* on each plot was recorded using a baited transect method was employed using crumbled sweet digestive biscuits as bait (Bourn pers. comm.). For each plot three baits were placed within each of three randomly located 5m radius sub-plots. Baits were left for 2-3 hrs before recording the number of ants.

### 2.3 Data Analysis

Data analysis were performed using IBM SPSS Statistics version 9. Relationships between variables were examined using Pearson’s Correlation test. Data were tested for linearity using the curve fit function in SPSS. Two-tailed statistical tests were performed with significance (*P*) assumed wherever *P* < 0.05.

### 3. RESULTS

Sites varied in their incidence of deer, silver studded blue butterflies and ants (Fig. 2a, 2b and 2c respectively). They also differ in important aspects of their vegetation structure particularly bare ground (Fig. 3a) mean vegetation height (Fig. 3b) and mean abundance of the main food plant *Erica tetralix*/*Calluna vulgaris* (Fig. 3c). At our study site at Arne *P. argus* was found on both wet and dry heath. However, it was much more abundant on wet heath that contains tall vegetation, which is largely dominated by *E. tetralix*, with patches of *M. caerulea* and some *C. vulgaris*. These sites also had high incidence of *Lasius* ants. The species of ant differed between dry and wet heath, on the dry heaths most ants were *L. alienus* whereas on the wet heath most were *L. niger*.

Tables 1 and 2 show the main results for plots on wet heath and dry heath, respectively. On wet heath habitat there is a significant, positive relationship between *C. nippon* (from pellet counts) and *P. argus* (*r* = 0.457, *P* = 0.033), but this relationship is not significant for dry heath plots. Counterintuitively, a higher frequency of *C. nippon* (from pellet counts) correlated significantly with a higher mean vegetation height (*r* = 0.512, *P* < 0.05) on wet heath. On dry heath, a higher frequency of *C. nippon* correlates to a lower mean vegetation height (*r* = -0.645, *P* <0.01), with more bare ground (*r* = 0.639, *P* = 0.01). On wet heath *L. niger* was found more abundantly in taller vegetation *r* = 0.620, *P* = 0.002, whereas on dry heath plots *L. alienus* was found more in lower vegetation height, *r* = -0.636 *P* = 0.011, with more bare ground *r* = 0.752 *P* = 0.001.

### Table 1. Correlations table for wet heath plots at Arne heathland (n = 22)

<table>
<thead>
<tr>
<th></th>
<th><em>C. nippon</em> pellets</th>
<th><em>L. niger</em> abundance</th>
<th><em>P. argus</em> abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>r</em></td>
<td><em>P</em></td>
<td><em>r</em></td>
</tr>
<tr>
<td>Vegetation volume (m²)</td>
<td>-0.279</td>
<td>0.208</td>
<td>-0.027</td>
</tr>
<tr>
<td>Vegetation height (cm)</td>
<td>0.512*</td>
<td>0.015</td>
<td>0.620**</td>
</tr>
<tr>
<td><em>Erica tetralix</em> (% cover)</td>
<td>0.129</td>
<td>0.568</td>
<td>-0.048</td>
</tr>
<tr>
<td><em>Molinia caerulea</em> (% cover)</td>
<td>0.262</td>
<td>0.240</td>
<td>0.115</td>
</tr>
<tr>
<td>Bare ground (%)</td>
<td>0.364</td>
<td>0.096</td>
<td>-0.049</td>
</tr>
<tr>
<td><em>C. nippon</em> pellets (n)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>L. niger</em> abundance (n)</td>
<td>0.327</td>
<td>0.137</td>
<td>-</td>
</tr>
</tbody>
</table>

*Significant P values, as tested by Pearson’s correlation (two-tailed probability), are given in bold. Significance *P* < 0.05, **P* < 0.01. Parameters are mean values*
Wet Heaths

In general, wet heath plots with a taller vegetation height had more *P. argus* recorded ($r = 0.475$, $P < 0.05$), with a significant positive correlation between abundance of larval food sources and *P. argus* abundance, $r = 0.472$, $P < 0.05$. The opposite can be said of habitat use of *P. argus* on dry heath plots where the butterfly was found in areas of shorter vegetation ($r = -0.690$, $P = 0.004$) and nearer to patches of bare ground ($r = -0.744$, $P = 0.001$). The available of butterfly food plant here was largely dominated by *Calluna* with frequent patches of *E. cinerea*.

On both wet and dry heathland habitats there was a strong positive correlation between Lasius ants and *P. argus* (wet heath plots $r = 0.607$, $P = 0.003$, dry heath plots $r = 0.835$, $P < 0.001$).

**Dry Heaths**

Fig. 2. Variation across the heaths in the mean incidence recorded for a) *C. nippon* deer, b) *P. argus* silver studded blue butterflies and c) *Lasius* spp. ants
Fig. 3. Variation across the heaths in a) their % cover of bare ground b) mean vegetation height c) mean abundance of Erica tetralix

Table 2. Correlations table for dry heath plots at Arne heathland (n = 15)

<table>
<thead>
<tr>
<th></th>
<th>C. nippon pellets</th>
<th>L. alienus abundance</th>
<th>P. argus abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Vegetation volume (m³)</td>
<td>-0.249</td>
<td>0.371</td>
<td>-0.331</td>
</tr>
<tr>
<td>Vegetation height (cm)</td>
<td>-0.645**</td>
<td>0.009</td>
<td>-0.636*</td>
</tr>
<tr>
<td>Erica cinerea (% cover)</td>
<td>-0.114</td>
<td>0.343</td>
<td>-0.015</td>
</tr>
<tr>
<td>Bare ground (%)</td>
<td>0.639*</td>
<td>0.010</td>
<td>0.752**</td>
</tr>
<tr>
<td>C. nippon pellets (n)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L. alienus abundance (n)</td>
<td>0.398</td>
<td>0.142</td>
<td>-</td>
</tr>
</tbody>
</table>

Significant P values, as tested by Pearson’s correlation (two-tailed probability), are given in bold. Significance *P < 0.05, **P < 0.01, ***P < 0.001. Parameters are mean values.
4. DISCUSSION

The aim of this study was to understand the impacts of *C. nippon* on the ecological drivers of *P. argus* at a site in Dorset where the butterfly exists on both wet and dry heath habitats. Overall our results show that grazing by *C. nippon* is associated with higher butterfly numbers. This agrees with findings from a study on heathlands in the Netherlands that *P. argus* was significantly more abundant under conditions of continous grazing by cattle compared to non grazing or summer-only grazing regimes [39].

Our results agree with previous work [6,40] that on Dorset's lowland heathland it is areas of wet heath dominated by *E. tetralix* that preferred by *P. argus*. However, this may change in future as it is thought that *P. argus* occupies a broader niche on the warmer sites in the south of England [1] and specific warmer microclimates are not as necessary as they are for *P. argus* inhabiting areas further north of its range. At our study site at Arne *P. argus* is predominantly found on wet heath where the vegetation grows in tall patches dominated by *E. tetralix* with a high frequency of the ant *L. niger*. This association with taller wet heath has also been found for *P. argus* at its southern European limit where taller vegetation is preferred which reduces ground temperatures [12]. On dry heath *P. argus* occurred most abundantly where the vegetation is grazed short; the heather is often in pioneer stage with plenty of bare ground, and there is a high occurrence of the ant *L. alienus*.

Fig. 4 summarises the impact of *C. nippon* on *P. argus* at Arne. It illustrates all significant correlations found in this study incorporating direct and indirect relationships between the deer and the butterfly.

Our findings confirm the key important of habitat provision for *Lasius* ants. *P. argus* is a obligate mutualist myrmecophile meaning the larvae are herbivorous and are able to pass plant nutrients to their attendant ants. Mutualistic interactions minimize costs, yet optimize the benefits occurring from this association. There is a high degree of intimacy, but ants never depend on caterpillar secretions for nourishment, whereas butterfly larvae may depend on their association with ants. Mutualistic ants were shown to be more important than nectar source density in determining persistence of *P. argus* [10] and the frequency of nests of *L. niger* was by far the major predictor of *P. argus* abundance and presence-absence in a study in Spain [41]. The butterfly was shown to be absent from sites with low frequencies of *L. niger* or *L. alienus*.

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![Fig. 4. *Cervus nippon*'s relationship with *P. argus* and its impacts, showing significant correlations found in this research.](image)

*Arrows indicate relationships, WET = wet heath, DRY = dry heath, +/- = positive/negative correlation, *= significance values where * P < 0.05, ** P < 0.01, *** P < 0.001*
even though these sites contained abundant host plants and favourable microclimates [12]. The populations of *P. argus* found on heathland must therefore choose locations where the black *Lasius* ants inhabit amid their food plant. An increase in *Lasius* abundance equates to an increase in the abundance of *P. argus*. At Arne it may also be the case that *Lasius* ant densities are higher on wet heath sites than they are on dry heath.

*Lasius niger* is characteristic of low-lying wet areas, that are well vegetated, especially with the grass *M. caerulea* and the heath *E. tetralix* [42]. In a study in Spain [41] almost all *L. niger* nests are found at the bases of shrubs, because roots provide suitable sites for nest-building in the sandy soils. Although ants' nests were not studied by us it is a possibility that *L. niger* prefer to nest where clumps of *E. tetralix* or other equally suitable vegetation is growing for similar reasons. On dry heath areas the ant *L. alienus* prefers a warmer microclimate with a lower vegetation height and more bare ground [43]. The markedly different behaviour of *P. argus* inhabiting wet and dry heath is due to the habitat preference of the mutualistic ants on each heath type. The specific habitat preferences for each species depend on the vegetation type and cover and also to a certain degree elevation above sea level.

On wet heath plots we found areas with a taller vegetation height appeared to be preferred by *P. argus*. This agrees with findings from a coastal site in north Wales where population density was higher in shrubby areas where a substantial and significant bias in roosting and mating was found to occur and *P. argus* density was shown to be higher in the vicinity of shrubs which were also used for resting, basking, mate location and shelter [44,45]. At Arne gorse *Ulex europaeus*, bracken *Pteridium aquilinum* and grasses (mainly *M. caerulea*) were found in plots with a higher concentration of *P. argus*. *M. caerulea* is a highly digestible species [46] that flourishes under grazing and also possibly through dung deposition which may create high nutrient concentrations which certain grasses favour over dwarf shrubs [47]. On wet heaths *M. caerulea* can quickly fill gaps in open spaces in the vegetation cover or heather canopy. This could lead to taller mean heights as the grass grows taller to compete for light [48] but, *M. caerulea* only increased significantly when moisture content exceeded 60% [49].

The presence of *E. tetralix* is an important factor for *P. argus* populations as it is the predominant food plant on wet heath plots. The lightly and regularly grazed heathers still provide new shoots of growth on which the caterpillar can feed on [50]. Grazing on dry heath plots maintains a young green canopy where self-shading from senescent leaves is reduced [51] thus enabling larvae to feed on suitable stems of the foodplant. On dry heath a short vegetation height with open patches of bare ground is preferred. These warmer microclimates may be favoured because they support high *Lasius* densities rather than because of any direct effect of temperature on the butterflies [12].

In summary, our study has shown that *C. nippon* graze areas of both wet and dry heathland and this is creating suitable habitat for both *L. niger* and *L. alienus* ant species. Grazing levels are not too high on either heath type despite high overall population densities of approximately 1 deer per ha. Our study agrees with previous findings [52] and show that this is because sika deer use dry heaths and other habitats more than wet heaths. The grazing by *C. nippon* creates open ground for ants and stimulates fresh growth of the larval food plant, whether that is *E. tetralix* (in wet heath plots), or *C. vulgaris* (in dry heath plots). Grazing also maintains a young green canopy where self-shading from senescent leaves is reduced. In doing so *C. nippon* creates a favourable environment for *P. argus*, therefore increasing not only the likelihood of the butterfly’s presence but also its abundance.

On dry heath *C. nippon* grazing creates open bare ground and shorter vegetation with shorter stands of heather. On wet heath areas deer prefer plots with taller vegetation as they use such plots for harbourage as well as food sources. In particular we often witnessed young calves hidden in taller wet heath. The wet heath plots were, fortuitously, not over-dominated by *M. caerulea* and so the light grazing is sufficient to control this and create light gaps for *Lasius* ants and fresh growth of *Erica tetralix*. Care must however be taken with that *M. caerulea* does not become over-dominant on the sites as a result of lack of grazing (Chambers et al., 1999). Similarly, any grazing regime using a wild herbivore such as Sika deer will need population monitoring and management to ensure that dry heaths are not over-used so damaged.
5. CONCLUSION

Overall our results indicate that grazing by *C. nippon* increased the abundance of *P. argus* and suggest that the causal relationship may be via *C. nippon* creating habitat suitability for *Lasius* ants more than by direct impacts on the larval food plants.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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