SELECTION EXPERIMENTS ON MELANISM IN *AMATHES GLAREOSA* ESP. (LEPIDOPTERA)

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INTRODUCTION

THE Noctuid moth *Amathes glareosa* Esp. is widely distributed throughout the Shetland Isles (Kettlewell and Berry, 1961). It has been established that at the north end of the islands 97 per cent. of the population are of the melanic form *edda*. By contrast on south Shetland Mainland, less than 60 miles away, the population is 98 per cent. of the light form. In the intervening region there is a steady frequency cline which is interrupted by a rather rapid change near the Tingwall Valley, which appears to act as a partial barrier. Fuller details of the two contrasting populations to the north and to the south must be recorded here.

1. THE STRUCTURES OF THE TWO POPULATIONS

(i) Unst, North Shetland

In 1959 a total of 2539 *A. glareosa* were examined by C. J. Cadbury. Of these 77 were *f. typica* (3.03 per cent.). This sample was smaller than that taken in 1960, because of an insufficient number of mercury vapour traps on which we depend for sampling. The total captures in each of these years, therefore, bears no direct relationship to the abundance of the species.

In 1960 10,650 *A. glareosa* were caught of which 294 were *f. typica* (2.76 per cent.) (text-fig. 1). In the first week 90 per cent. of the *A. glareosa* were males but at the end of the season a higher proportion were females. Table 1 shows nightly catches for the season along with the percentage of *f. typica*. There is some evidence that the frequency of *f. typica* in the north increases throughout the hatching period. This will be discussed later.

(ii) Dunrossness, South Mainland

The structure of the population is very different here. Of 5013 *A. glareosa* which were captured in 1960, 4913 were *f. typica* (98 per cent.). There was no evidence that *f. edda* appeared earlier than *f. typica* in these figures, nor would it be expected if predation is relaxed in south Shetland, since there would then be no selection in favour of the later emergence of either form.

This situation of two contrasting populations appeared to offer exceptional opportunities for analysis by experimentation in the field. With the help of a team already trained in the special techniques, I decided to undertake extensive mark-release-recapture experiments both in north and south Shetland, the two to run concurrently. By

choosing the opposite ends of the Shetland Isles as centres of our experimental releases where the frequencies of the two forms showed their greatest contrasts, I had hoped to be able to evaluate the results of selective predation in the same way as had been possible in industrial melanism. The method employed was to catch and mark large numbers of each form at both locations. A proportion of the commoner

TEXT-FIG. 1.—Wild captures of the two forms of *Amathes glareosa* taken in mercury vapour traps, Unst 1960 (continuous line *f. edda*).

of the two forms was then transported as rapidly as possible to the opposite end of the islands. In view of the fact that the distances involved were small, it has been assumed that each phenotype, *regardless of origin*, was identical with another of the same form in regard to its behaviour. Further work will confirm or deny this.

Unfortunately in carrying out a design of experiment as complicated as this we met with considerable difficulty. There are two sounds to be negotiated, firstly between the islands of Unst and Yell, and secondly between Yell and north Shetland Mainland. Nevertheless, with the collaboration of the Shetlanders, a parcel of live insects was sent each day from north to south and another from south to north. This demanded the daily use of four separate omnibus services and two

ferries in each direction. The specimens were contained in pill boxes, 2 in a box, and it is fair to the Shetlanders to put on record that on no single occasion were any of the 44 parcels either damaged or lost. All the moths arrived in excellent condition and by this means we had a plentiful supply of the rarer of the two forms at each of the opposing centres of mark-release. Because of the journey, however, 36 hours elapsed between the catching of the moths and their subsequent release. We were punctillious throughout therefore to ensure that the locally caught specimens were similarly incarcerated for 36 hours, 2 to each pill box. This shuttle service continued daily (except Sunday) from 14th August till 5th September.

2. MARKING AND RELEASING TECHNIQUES

Marking techniques were modelled on those devised by Dowdeswell, Fisher and Ford (1940). I have previously used modifications of these on a number of Lepidopteran species with satisfactory results. *A. glareosa*, however, presented particular difficulties. A small scuttling Noctuid moth, whose hindwings project almost as far as the forewings, and which at the same time are plicated like a fan, was found to be very different from those species with which I have previously worked. The lethargy of *Biston betularia*, the surface area of *Lasiocampa quercus*, and the convenient white spotting of *Panaxia dominula* were all absent. Nevertheless we eventually developed a technique for successful marking without the use of an anæsthetic which at one time we thought would be necessary. This depended on a method of handling the moth with forceps in such a way that the wings were dorsi-flexed and ensuring that they were not stuck together after applying the paint spot.

Using a combination of the undersides of four wings, and five colours, we were able to identify twenty individual markings, and we found this adequate for the life-span of the species. In 1959 a small pilot experiment had been undertaken on Unst (Site 1), but because of an inadequate knowledge of the natural history of the moth, an insufficient supply of *f. typica* from south Shetland was available. In 1960 we commenced mark-release experiments on the same releasing site (Site 1), but this was abandoned after three days because, unlike 1959, there was no evidence of birds feeding in the area. The new site chosen (Site 2) was separated from the first by a half-mile stretch of sea. It was situated on the side of the hill of Muckle Heog to the north of Baltasound. Geologically this area consists of serpentine rock and as such is a specialised biotope maintaining many rare species of small plants (Spence, 1957). Due to the toxic nature of the soil the vegetation is sparse and the peat-coloured soil is shallow, though no depth of peat is present.

On our first visit in 1959 we arrived on 1st August. Subsequently we found *A. glareosa* extremely common here. Furthermore, birds were active on the ground not only by day but throughout most of the night. After mid-August, however, the hours of darkness rapidly increase

at this latitude, so that in 1960 when we arrived two weeks later we observed no twilight activity by birds whatever. Nor did we record any at full moon which was on 5th September. Diurnal feeding, however, was very much in evidence on Site 2.

TABLE I

Wild population captures for Amathes glareosa and its melanic form edda, Unst, N. Shetland, 1960

	Date	Phenotype		Total	Per cent. <i>typica</i>
		<i>Typica</i>	<i>Edda</i>		
<i>Site 1</i>	13.8.60	0	3	3	...
	14.8.60	15	835	850	1.765
	15.8.60	19	611	630	3.016
	16.8.60	19	465	476	3.311
	17.8.60	18	629	647	2.782
	18.8.60	12	485	497	2.415
<i>Site 2</i>	19.8.60	54	1,792	1,846	2.925
	20.8.60	14	743	757	1,849
	21.8.60	9	484	493	1.826
	22.8.60	6	166	172	3.488
	23.8.60	43	1,530	1,573	2.734
	24.8.60	37	1,111	1,148	3.223
	25.8.60	7	225	232	3.017
	26.8.60	3	110	113	2.655
	27.8.60	4	88	92	4.34 ⁸
	28.8.60	13	320	333	3.904
	29.8.60	7	160	167	4.192
	30.8.60	3	58	61	4.918
	31.8.60	5	68	73	6.849
	1.9.60	0	61	61	...
	2.9.60	1	41	42	2.381
	3.9.60	4	99	103	3.883
	4.9.60	2	54	56	3.571
	5.9.60	0	5	5	...
	6.9.60	4	30	34	11.765
	7.9.60	2	121	123	1.626
	8.9.60	1	62	63	1.587
		294	10,356	10,650	2.761

Total captures	= 10,650
Total <i>typica</i>	= 294
Total <i>edda</i>	= 10,356
Average percentage of <i>f. typica</i>	= 2.761

This new releasing ground was approximately one-quarter square mile in extent and from 18th August till 5th September large numbers of *A. glareosa* were released daily, each form being as near to equality as possible. Because of the eccentricities of the omnibus services the release was not practicable before 4 p.m. The time available for predation on the first day was therefore limited to a few hours. After release the behaviour of both forms followed a definite pattern. Both fed for a short period on heather flowers and then took up their

SELECTION FOR MELANISM

419

1960 Site 1	Release	Day														Total recaptures both forms NOT recaptured
		Day														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E
14.8	46	3	0 0	0 0	3 0										3 0	46
15.8	95	295	2 0	1 4	2 5										5 9	376
16.8	106	199	1 2												1 2	302
Total	247	497														724
Site 2																
18.8	95	182	10 4	3 6	2 0	0 1	3 0	1 2	0 0	0 0	0 0	0 0	0 0	0 0	19 13	245
19.8	223	229	5 6	1 3	1 0	4 4	2 5	0 0	0 0	0 0	0 0	0 0	0 0	0 0	13 18	421
20.8	179	230	0 0	0 1	2 7	4 3	0 0	0 1	0 0	0 0	0 0	0 0	0 0	0 0	8 13	388
21.8	193	245	0 0	6 8	5 9	0 2	0 2	0 1	0 0	0 0	1 0	0 0	0 0	0 0	12 23	403
22.8	149	150	13 7	3 6	1 0	0 2	0 1	0 1	0 0	0 0	0 0	0 0	0 0	0 0	17 18	264
23.8	7	9	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 2	13
24.8	223	213	3 2	1 1	1 1	0 3	0 0	0 0	0 2	0 1	0 0	0 1	0 0	0 0	5 12	419
25.8	155	243	0 0	1 2	1 6	0 1	0 1	1 0	0 0	0 0	0 0	0 0	0 0	0 1	4 11	383
26.8	70	81	1 0	0 3	0 0	0 1	0 1	0 0	0 0	1 0	0 0	0 0	0 0	0 0	2 5	144
27.8	18	36	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 1	0 0	0 0	1 2	51
28.8	93	86	0 2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 2	176
29.8	176	166	0 1	3 4	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 1			4 7	331
30.8	148	102	0 0	0 0	1 2	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	2 3	245
31.8	74	35	0 0	0 0	1 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	1 0	108
1.9	81	44	0 0	0 0	0 0	0 0	0 0	0 1							0 1	124
2.9	51	32	1 0	0 0	0 0	1 0	0 1	1 0							0 1	79
3.9	52	40	0 0	0 0	0 0	0 0	0 0								0 1	91
4.9	52	83	0 0	1 0	0 0	0 0	0 1								1 10	134
5.9	45	54	0 0	1 0	0 0	0 0	0 0								1 0	98
Totals	2084	2260	34 25	20 34	16 26	11 19	6 11	3 6	2 3	1 2	0 1	1 1	0 1	0 0	95 132	4117
	4344		1	2	3	4	5	6	7	8	9	10	11	12	13	14
																4344

Typical days = 256

Edda days = 440

characteristic resting positions on the ground and from then on rarely moved till dark.

Around this releasing ground and at distances of approximately 200 to 500 yards we had three concealed 125-watt mercury vapour traps. These were turned on each evening at early dusk and off at dawn. The highly successful type of trap we have now developed

TEXT-FIG. 2.—Histograms of marked recaptures of both forms of *Amathes glareosa* showing the number of days' survival in the wild population (from 19 separate releases), Unst 1960.

captures, we believe, random samples of the insects which are in flight. A similar design of sampling was employed at each of the releasing sites.

3. RELEASE DATA

We are here concerned with the recapture results of three release experiments undertaken in this way in 1960; two on Unst on north Shetland and one in the south Mainland. On Unst 90 per cent. of the *f. typica* released came from south Shetland: in Dunrossness 92 per cent. of *f. edda* were imported from Unst.

Unst Site 1: Total release 744 *A. glareosa* (*edda* 497; *typica* 247).

This site was abandoned after three days for the reasons given above.

Unst Site 2: Total release 4344 *A. glareosa* (*edda* 2260; *typica* 2084).

Dunrossness, south Shetland: Total release 2144 *A. glareosa* (*edda* 961; *typica* 1183).

Tables 2 and 3 show the distribution of subsequent recaptures and text-fig. 2 depicts the histograms of recaptures of each form from Unst

TABLE 3
Release-recapture figures of both forms of *Amathes glareosa*, South Mainland, 1960

Release date	Release	No. of days between release and recapture											Recaptures Total		Unrecaptured moths		
		1	2	3	4	5	6	7	8	9	10	11	T	E	T	E	Total
		T	E	T	E	T	E	T	E	T	E	T					
20.8.60	60	7	1	2	1	2	1	...	...	...	...	...	11	4	51	92	143
21.8.60	170	1	0	3	0	...	...	...	...	...	...	...	9	0	161	1	162
22.8.60	159	1	2	0	1	...	...	...	...	0	0	0	5	11	154	156	210
23.8.60	13	0	0	0	0	1	0	...	...	...	...	...	2	0	11	1	12
24.8.60	70	0	0	0	1	0	0	0	...	...	...	...	2	3	68	102	170
25.8.60	172	1	0	0	3	0	0	0	...	...	...	...	2	4	170	152	322
26.8.60	72	0	0	0	0	0	0	0	...	...	...	...	1	3	71	68	139
27.8.60	5	1	0	0	1	...	...	...	...	...	...	...	1	2	4	32	36
28.8.60	1	...	...	...	...	...	...	...	...	...	...	...	0	0	1	3	4
29.8.60	97	0	0	2	4	0	0	1	...	1	...	...	7	16	90	138	228
30.8.60	130	0	0	2	1	2	2	...	...	...	...	...	6	3	124	49	173
31.8.60	46	1	0	...	...	...	...	...	...	...	...	...	1	0	45	25	70
1.9.60	39	0	0	0	0	0	0	...	...	...	...	...	2	1	37	44	81
2.9.60	45	0	0	0	0	0	0	2	...	...	...	...	3	2	76	38	114
3.9.60	79	0	0	0	0	2	2	...	...	...	...	...	0	0	19	3	22
4.9.60	40	1	...	...	...	...	...	...	...	...	...	...	0	0	48	5	53
5.9.60	5	0	0	0	1	...	...	...	...	...	...	...	0	1	1	2	3
	1183	13	3	9	12	8	3	4	6	4	5	0	52	50			
		1	2	3	4	5	6	7	8	9	10	11					

TABLE 4

Observed and expected recaptures of both forms of Amathes glareosa for day 1, day 2 and for the total period, together with variance

1960 North End Site 1 (Unst, Shetland)	Day 1			Day 2			Total recaptures all days		
	Obs. edda	Exp. edda	Var.	Obs. edda	Exp. edda	Var.	Obs. edda	Exp. edda	Var.
14th August	0	...	...	0	...	...	Site abandoned after 3 days		
15th	0	1.513	0.368	4	3.782	0.912			
16th	2	1.957	0.676	0	...	...			
Total Site 1	2	3.470	1.044	4	3.782	0.912			
Site 2									
18th August	4	9.199	3.006	6	5.913	1.969	13	21.025	6.401
19th	6	5.573	2.689	3	2.027	0.993	18	15.706	7.233
20th	0	...	...	1	0.562	0.245	13	11.809	4.915
21st	0	...	...	8	7.831	3.348	23	19.578	7.955
22nd	7	10.033	4.681	6	4.515	2.190	18	17.559	7.751
23rd	2	1.125	0.459	0	...	...	2	1.600	0.640
24th	2	2.443	1.238	1	0.977	0.499	12	8.305	4.092
25th	0	...	...	2	1.832	0.710	11	9.158	3.441
26th	0	0.536	0.249	3	1.609	0.736	5	3.755	1.671
27th	1	1.353	0.436	0	...	...	2	2.000	0.642
28th	2	0.961	0.496	0	...	...	2	1.441	0.740
29th	1	0.485	0.250	4	3.398	1.718	7	5.339	2.667
30th	0	...	...	0	...	...	3	2.040	1.188
31st	0	...	...	0	...	...	0	0.321	0.218
1st September	0	...	...	0	...	...	1	0.352	0.228
2nd	0	0.386	0.237	0	...	...	1	1.542	0.913
3rd	0	...	...	0	...	...	1	0.435	0.246
4th	0	...	...	0	...	...	0	0.615	0.237
5th	0	...	...	0	...	...	0	0.545	0.248
Total Site 2	25	32.074	13.741	34	28.664	12.408	132	123.125	51.426
							Diff. : 8.875 ± 7.1712 (Standard error) $1.24 = P > 0.21 < 0.22$ = 7 per cent. diff. in favour of <i>f. edda</i>		

releases site 2. A full analysis of the south Shetland experiment is not given because both the release techniques and the site chosen were considered unsatisfactory.

4. ANALYSIS OF RECAPTURE DATA

On comparing the histograms of the two forms for the main release in the north, in which recaptures are recorded according to the number of days' survival of each form in the wild, two main points stand out. Firstly there is a deficiency of *f. edda* recaptures on the first day. On the other hand the histogram for *f. typica* has a distribution according

to expectation. In all release experiments more recaptures are to be anticipated on the day immediately after release than on subsequent days because of depletion by predation, dispersal or death from other causes. The deficiency of first day recaptures of *f. edda* is therefore

TABLE 4—continued

1960 South End (Dun- rossness, Shetland)	Day 1			Day 2			Total recaptures all days		
	Obs. <i>edda</i>	Exp. <i>edda</i>	Var.	Obs. <i>edda</i>	Exp. <i>edda</i>	Var.	Obs. <i>edda</i>	Exp. <i>edda</i>	Var.
20th August	1	4.861	1.822	1	0.608	0.238	4	9.114	
21st	0	0.006	0.006	0	0.012	0.012	0	0.053	
22nd	2	1.537	0.745	4	3.586	1.717	11	8.196	
23rd	0	...	...	0	0.071	0.066	0	0.143	
24th	0	...	...	0	0.600	0.240	3	3.000	
25th	0	0.476	0.249	0	0.476	0.249	4	2.854	
26th	0	...	...	2	1.490	0.739	3	1.986	
27th	0	0.872	0.112	1	0.872	0.122	2	2.615	
28th	0	...	...	0	...	...	0	...	
29th	0	...	...	4	3.681	1.394	16	14.112	
30th	0	...	...	0	0.571	0.406	3	2.571	
31st	0	0.352	0.228	0	...	...	0	0.352	
1st September	0	...	...	1	0.536	0.249	1	1.607	
2nd	0	0.336	0.223	0	...	...	2	1.681	
3rd	0	...	...	0	...	...	0	...	
4th	0	...	...	0	...	...	0	...	
5th	0	...	...	1	0.750	0.188	1	0.750	
Total South Shetland	3	8.440	3.385	14	13.253	5.610	50	49.034	
Combined totals of 3 experiments	30	43.984	18.170 P = 0.001	52	45.699	18.930 P = 0.15	Therefore no evidence of selective predation in figures		

There is no heterogeneity for day 1 recaptures in the three release experiments, nor for day 2. The results can therefore be combined for each day separately.

For day 1 recaptures $P = 0.001$

For day 2 recaptures $P = 0.15$

Therefore there is a highly significant deficiency of *f. edda* recaptures on day 1, compared to *f. typica*.

quite exceptional and was unexpected. If this departure from expectation was significant it could be of importance.

A second point shown by the histograms is that the total *f. edda* recaptures (assuming equality of release) was greater than that of *f. typica*.

5. DISCUSSION 1

A. Evidence of a behavioural difference in the wild

A comparison of the proportions of recaptures of the two forms on day 1 shows that there is a deficiency of *f. edda* on that day and that the results are homogeneous in all three experiments (table 4). If we

consider the results of our main experimental work in the north (Unst site 2), we can calculate the mean life-span of both phenotypes after release, for *f. edda* 3.41 days, for *f. typica* 2.69 days. Assuming these to be constant for each form the expected number of recaptures can be assessed for each day. Hence we can test whether the observed recaptures deviate from the expected. They do not (for *edda* $\chi^2_{(13)} = 12.29$; for *typica* $\chi^2_{(11)} = 1.79$) except for the *edda* recaptures for the first day after release on which there is a significant deficiency of recaptures ($\chi^2_{(1)} = 4.85$; $P = >0.02, <0.05$).

Another method of considering this is to take the total recaptures of both forms on both day 1 and day 2 and to compare each of these with the number expected along with its variance (tables 4 and 4a). This method is based upon a technique developed by C. A. B. Smith

TABLE 4 (a)

A comparison of day 1 and day 2 recaptures from three mark-release-recapture experiments, Shetland, 1960 (summary of table 4)

	Day 1	Day 2
Observed <i>edda</i>	30	52
Expected <i>edda</i>	43.984	45.699
Variance	18.170	18.930
	$P = 0.001$	$P = 0.15$

for sibship studies in duodenal ulcers (Clarke *et al.*, 1956). In this we have had to make the assumption that the two forms have equal death rates. This is probably near the truth for the first day after release.

When the recaptures for day 1 and day 2 for all three experiments are considered in this way, the probability of such a deficiency of *f. edda* occurring on the first day by chance is 1 in 1000. An explanation of this is unlikely to be accounted for by selective predation in favour of *f. typica* seeing that this did not occur on subsequent days. Furthermore, because of the late hour of release on the previous day, little, if any, predation could have actually taken place. This immediately suggested that our method of sampling was in error and that the absence of *edda* on day 1 might reflect a habit difference. It seemed likely in fact that *f. edda* (of local Unst origin) took flight less frequently than *f. typica* (from south Shetland) and that this habit difference was reflected in our trap samples which depended on taking random moths on the wing.

It must here be pointed out that flight in the Shetland Isles in common with other small islands, is a hazardous occupation for most insects, particularly if undertaken near the coast. It will be recalled how on certain islands in other parts of the world apterous species have developed because of this. The average wind strength throughout

the year in north Shetland is similar to that at the top of Ben Nevis. Nevertheless even under these conditions there must be compensating advantages for flight such as a capacity for finding a mate, an opportunity for feeding or ovipositing on suitable plants, and an assurance of adequate gene-flow. Maximum land dispersal with minimum loss through drowning must in fact conflict in most island species.

We are able to provide further evidence that a habit difference between south Shetland *f. typica* and northern *f. edda* exists. Equal numbers of both forms (25 of each) were marked and released into an area of approximately 10 square yards at 8 p.m. All the moths took up resting positions after having previously fed. From then till dark I was in a close position to observe them and see that no bird predation took place. The first sign of activity took place at late dusk (9.55 p.m. G.M.T. 18th August). Fifteen *f. typica* were seen to fly before 11 p.m., but only 5 *f. edda*. This could be accounted for because of the greater ease of seeing the light coloured *f. typica* on the wing. However, using a torch a count of 12 *edda* to 3 *typica* ($\chi^2_1 = 4.3$) was made at midnight as they fed or rested on the heather. It was apparent that most of the *f. typica* had flown. Furthermore, twenty-four hours later on the following evening 5 marked *edda* were found but no *f. typica* in spite of this form being so much more conspicuous. It is hard to construe these observations in any way other than that under certain conditions *f. edda* takes flight less frequently than *f. typica*, but it must be remembered that the majority of our *f. typica* come from south Shetland.

Independent evidence of a habit difference was obtained by each of us who were responsible for undertaking the daily releases for our main experiments. On being thrown into the air *f. typica* would frequently fly a short distance before settling: *f. edda* was much more lethargic and usually fell to the ground.

Evidence that this habit difference may be associated with the two forms is provided from our sample data from the Tingwall Valley which is situated in the centre of the Mainland barrier zone (Kettlewell and Berry, 1961). In contrast to our usual finding that the frequencies of our samples vary but little from one night to the next, here on the north side of the valley, nightly frequencies fluctuated widely. Heterogeneity by the contingency test is $\chi^2_{(4)} = 13.10$. There is some evidence also that on many nights the frequency of *f. typica* on the north side was inversely proportional to that on the south. Though these observations were made at a period when the species was diminishing in numbers and the samples were therefore small, the most reasonable explanation is that the fluctuations were brought about by a differential flight habit between the two forms. The situation at the Tingwall Valley is that the population to the south is largely composed of wild type. To the north, however, the melanic form rapidly becomes more common. Part of the 1961 expedition will be attempting to find out more about the habits of the two forms in this most interesting area. We have, however, already provided

evidence that different behaviour patterns occur within the species in the wild.

This is the first occasion on which a habit difference has been demonstrated in the wild by mark-release methods in a polymorphic insect. Hovanitz (1948), by other methods demonstrated an activity difference in the white mutant female of *Colias* butterflies. It is likely that behavioural differences of this kind will be found much more commonly in future and that they will be seen to contribute materially to most balanced polymorphisms.

Mechanism and origin of habit difference. A differential flight activity could be brought about by two distinct mechanisms. Either activity could be controlled by genetic factors or if associated with *f. edda* it could be the direct consequence of the physical difference between black and white bodies. If genetic in origin the behavioural difference could be pleiotropic or it could be controlled by one or more modifying genes which have become linked to the gene responsible for the pigmentation of *edda*. Alternatively it could have arisen independently of either form at the opposing ends of a population subject to intensive selection under high wind conditions on small islands and headlands, and where gene-flow was limited between the north and south ends. It has been pointed out that habit differences are more likely to be found in long-standing balanced polymorphisms than in the comparatively recent one of industrial melanism (Kettlewell, 1961).

If decreased flight activity is associated with the melanic *f. edda*, a simple physical phenomenon could account for this as a direct consequence of black colouration. It is necessary for Noctuid moths to vibrate their wings for a considerable period of time before taking flight and this is in order to increase their internal body temperature. The temperature on Unst at night is fairly constant in the month of August and seldom rises above 55°F. It is possible that on colder nights the heat lost from the black surfaces of *f. edda* is more rapid than the heat produced and that this does not take place in the same way with the light coloured *f. typica*. We intend to check this by using thermo-coupling in the near future.

B. Evidence of a different time of emergence

If the frequencies of *f. typica* in the daily samples of the wild population on Unst shown in table 1 are graphed (text-fig. 3) it will be seen that there is a steady increase of this form throughout the period of hatching.

After the end of August it is likely that the majority of *A. glareosa* pupæ have emerged, the population density is in a decline, the samples get smaller and *f. typica* will be disappearing from the population, due to selective predation, more rapidly than *f. edda*. These facts probably account for the drop in the *typica* frequency after 31st August. There are proportionately more *f. typica* in the population in the

week after peak emergence than before. Williamson (1956) has observed on Fair Isle that *f. edda* (where the phenotype frequency is 30 per cent.) appeared in advance of *f. typica*. Three *edda* were caught on 4th August 1955, but no *f. typica* were taken until one week

TEXT-FIG. 3.—Graph showing daily percentage of *f. typica* in the wild population on Unst 1960.

later. If the wild type occurs more frequently in samples collected later in the hatching period there are three possible explanations:

1. That the figures directly reflect a deficiency of *f. typica* in the earlier part of the season as the result of selective crepuscular predation which is possible at this time, but not later. Because of the longer hours of darkness which come on rapidly towards the end of August, crepuscular predation may be relaxed. We have been able to show that under conditions of twilight *f. typica* is at a cryptic disadvantage. The average maximum distance that three of us could see *f. typica* in the twilight or full moon, as it fed on heather flowers, was 12 feet: for *f. edda* it was 2 feet.
2. That because of the increased number of hours of darkness late in the season, *f. typica* which may fly more freely than *f. edda*, has greater opportunities than *f. edda* of coming to light traps.
3. That *f. typica* emerges later in the hatching period and that this is controlled genetically and adjusted by selective pressure. Natural selection would favour those *typica* which hatched later and hence were protected by darkness.

In regard to explanations 1 and 2 our recapture data do not show that a larger proportion of marked *f. typica* disappeared from our releases in the first half of the experiment than in the second. Nor do they provide evidence that marked *f. typica* were recaptured at light more freely later in the season. In fact we recaptured 6.25 per cent. of marked *typica* during the first nine days and 1.89 per cent. of our releases during the last ten. It is likely, therefore, that in Unst this seasonal increase in the frequency of *typica* is also a habit difference and that the majority of *f. typica* pupæ hatch at a somewhat later date than those of *f. edda*. Selection for this may have taken place only on those islands which are subjected to intensive bird predation. It is therefore more likely to be observed in populations maintaining a moderately high frequency of *f. edda*.

6. DISCUSSION 2

A. Evidence of predation and of differential survival in the wild

Direct observation on predation. As early as 1876 Buchanan White (1876) recorded that gulls and other birds scouted moths on the wing in twilight in Shetland. More specifically referring to *Hepialus humuli* (figured Kettlewell, 1961a), he states that the white typical forms must be very conspicuous in flight and that the darker forms would be at an advantage under the prevailing conditions. Similar observations have been made by Griffith (1929) who recorded gulls hawking male *H. humuli* for twenty to thirty minutes at dusk. Venables and Venables (1955) also comment on how the gulls appear quite suddenly as soon as the *H. humuli* flight commences and leave after half an hour when it is over. In 1960 we observed the common gull taking on the wing both forms of *Arenostola pygmina* Haw. which flies in the late afternoon. In 1959 in early August we saw many species of birds feeding on the ground both by day and during the long twilights. We had anticipated that *A. glareosa* would be taken on the wing in the same way as *H. humuli* but saw no evidence of this. To test this we impaled equal numbers of both forms alive on small fish hooks and these were allowed to fly at dusk suspended from very fine nylon stretched out over distances of approximately 100 yards. C. J. Cadbury observed that neither form was predated. He also noted that both forms of *A. glareosa* had whitish hindwings and that they exposed them in flight. This suggested that it was unlikely that aerial predation comparable to that which is known to take place in *H. humuli* was the main selective force at work. If predation in flight was excluded there remained two other vulnerable periods in the course of a day. The moth could be eaten (i) whilst at rest on the ground by day or (ii) whilst feeding on heather flowers or ovipositing in the twilight.

(i) *Evidence of A. glareosa predation by day.* We constantly saw birds feeding by day on the hillsides through binoculars. The most frequent

species were the common gull *Larus canus* L., the wheatear *Enanthe ænanthe* L., the peewit *Vanellus vanellus* L., the golden plover *Charadrius apricarius* L. and the starling *Sturnus vulgaris* L., the last two species frequently being in flocks. The common gull, up to 50 per cent. of whose diet has been shown by Spärck (1950) to consist of insects, was because of its size likely to be a formidable predator. Breeding common gulls are common throughout the north Shetland Isles but probably less so in the south (Venables, personal communication). They are also augmented by migratory flocks from the north in mid-August (Venables and Venables, 1955).

I therefore decided to shoot a minimum number of gulls during daylight. This was limited to five in all, three in August and two subsequently, both of which had not been feeding and had empty stomachs. Of the three, one was killed on our release ground and the two were half a mile from it. Of the latter, gull 1 contained 8 *A. glareosa*, one of which was *f. typica*. Three specimens of *Cerapteryx graminis*

TABLE 5

Observed feeds per minute of four common gulls, Unst

Gull 1	.	.	20 feeds in 7½ minutes
Gull 2	.	.	3 „ „ 2 minutes
Gull 3	.	.	6 „ „ 3½ minutes
Gull 4	.	.	5 „ „ 5½ minutes

This shows an average of one feed per half minute.

were also among its stomach contents (plate I, fig. 1). Gull 2 contained 10 *A. glareosa* of which one also was *f. typica*. Two in 18 (11 per cent.) *A. glareosa* were therefore of the white form; the frequency in the local population was 2.76 per cent. The gull which was shot on the releasing ground contained one marked *typica* previously released by us. On being shot and held upside down the gulls regurgitated the insects which were at the time unrecognisable (plate I, fig. 2). These were cleaned in spirit by Graham Phillips and he was able to show that shortly afterwards both forms were easily identified; the black ventral surface of the abdomen of *f. edda* was distinguishable in the most mutilated specimens. Furthermore he observed the feeding habits of the gulls. They worked individually at a considerable distance apart, and as many as 25 could be seen at a time pacing the short heather on the hillside. He noted the number of feeds per gull per minute for birds which were actually feeding and not preening or resting. Table 5 gives the number of feeds for four common gulls under observation.

As the stomach contents contained by volume 60 per cent. of *A. glareosa* it is likely that during this period of the year this species is one, if not the main, food source of the common gull. Because of its size, many ounces per day must be eaten by each gull. Their efficiency in finding them astonished us because of the cryptic perfection of the moths, particularly that of *f. edda*. We ourselves succeeded in discovering only one *f. edda* at rest in the wild, though we were able to

follow many hundreds of released ones to their final resting positions (figured Kettlewell, 1961). Phillips also examined the stomach contents of wheatear, peewit and starling but no individual Lepidoptera were recognisable. However, under the microscope he showed that the stomach contents contained large quantities of Lepidopterous scales. As *A. glareosa* was the commonest species of moth, it is reasonable to assume that this species contributed to them.

It is certain that throughout the summer a high predation pressure on Lepidoptera is maintained by indigenous birds and that in August and September diurnal predation is increased in North Shetland due to the arrival of migrating flocks. These are unlikely to affect the south Shetland Mainland to the same extent. Under these conditions selective visual predation must be intense, particularly in the north.

(ii) *Evidence of A. glareosa predation by night.* In 1959 we observed birds, in particular the common gull and wheatear, feeding on the ground until late twilight during the first half of August. It is likely, especially in warm weather, that *A. glareosa* comes up and feeds on heather flowers during the long period of dusk. We have provided evidence (*vide ante*) that under these conditions *f. typica* would be at a greater cryptic disadvantage. During feeding, both forms frequently sit upside down beneath heather sprigs and, as distinct from *f. typica*, the black ventral surface of the abdomen of *f. edda* serves to make this form less conspicuous. We have as yet no evidence on the degree of crepuscular predation that takes place in this way, but it is our belief that, particularly when full moon falls in the first half of August, it is likely to be heavy. Extensive selective predation in fact may take place both by day and by night in certain years and under certain conditions.

B. Evidence of selective predation from mark-release-recapture data

We have shown from our day 1 and day 2 recapture data that a flight habit difference exists between southern *f. typica* and northern *edda*. This being so it is impossible in our present experiments to differentiate the results of increased dispersal from those of predation. Nevertheless the one must bear a relationship to the other. Under weather conditions such as those experienced on Shetland an increased flight dispersal on small isles and headlands must frequently lead to death from drowning. This situation certainly applies to our releasing site (2) on Unst where the sea lies a few hundred yards to the east. It is therefore worth while considering our recapture data in this light. On Unst a total of 4344 *A. glareosa* (*edda* 2260; *typica* 2084) were marked and released. Of the *f. typica*, approximately 90 per cent. had their origin in south Shetland. In spite of the deficiency in *f. edda* recaptures for day 1 the total recaptures were 132 *f. edda* to 95 *f. typica*. A chi-squared test for significance cannot, however, be directly applied to these figures because an equal number of releases was not undertaken on each day, but it can be applied to the

two expecteds, *i.e.* 123.125 and 103.875 which gives $\chi^2_{(1)} = 1.40$, $P > 0.2 < 0.3$.

The recapture figures reflect a total of 440 days' survival for *f. edda* in the wild to 256 for *f. typica*. These recapture samples are, however, biased by small numbers of individuals which had survived for a long period of time, and this method of comparison is not therefore strictly valid.

A more refined method is to take the daily recaptures of both forms and compare these with the number expected, along with their variance using Smith's method (Clarke *et al.*, 1956). Table 4 shows that 132 *f. edda* were recaptured and 123.125 expected (a variance of 51.426, $P = > 0.21$ and < 0.22). There was not therefore a significant excess of *f. edda* recaptured. On the other hand, it suggests a 7 per cent difference in favour of *f. edda* on Unst, though because of the size of the variance this could be as high as 17 per cent advantage or as much as 5 per cent disadvantage. The size of the wild population into which we fed our marked individuals was too large for a significant selective predation difference to be shown by these methods, nor would it be easy to differentiate the dispersal of *f. typica* from selective predation against it.

By contrast the mark-release-recapture results in south Shetland, where no visible evidence of bird predation was seen, showed no differential survival. In releases totalling 2144 *A. glareosa* (*edda* 961; (approximately 92 per cent imported from Unst); *typica* 1183) observed *edda* recaptures were 50, expected 49.034. In the future therefore the answer to selective bird predation must depend, not on mark-release-recapture data, but on analyses of bird stomach contents, and for this the common gull has been shown to be the most suitable species.

7. CONCLUSIONS

The melanic form of *Amathes glareosa*, *f. edda*, is found at varying frequencies not only throughout Shetland and Fair Isle but also on the Orkneys. The polymorphism is likely therefore to be of considerable age and must be maintained by natural selection. A balance of advantages and disadvantages exists between the wild type and the melanic *f. edda*. By analysis and observation in the field we have been able to provide evidence as to what some of these are. The one gene difference between the two forms is responsible for the substitution of the normal disruptive pattern found throughout its range elsewhere, by a cryptic one of high efficiency for resting on the background of peat which is found throughout the northern half of Shetland. Under conditions of high selective pressures and in latitudes which permit many hours of both daylight and twilight predation, efficient camouflage has a priority over other demands.

Diminished flight activity, which may or may not be associated with *f. edda*, would also conserve loss through wind dispersal on small islands. On the other hand the advantages of flight and the normal

disruptive pattern in south Shetland, outweigh their disadvantages in north Shetland. This may in part be brought about by a relaxation of bird predation here, in a district of more cultivation, less peat-hag and with fewer small islands and headlands. In particular the common gull and also the vast flocks of immigrant birds from the north which arrive in north Shetland during the months of August and September are less frequent in the south. In Unst (and also Fair Isle), where gull predation is high, there is evidence that the light coloured *f. typica* emerge later in the hatching period than *f. edda*. The longer hours of darkness which rapidly come on later in the season, and the shorter periods of twilight, give more protection to this form, which we have shown is most conspicuous at dusk. Natural selection would therefore favour those *f. typica* which hatched later.

8. SUMMARY

1. By observation and experimentation an attempt has been made to analyse the relative advantages and disadvantages of melanism of non-industrial origin.

2. Mark-release-recapture experiments were conducted in both north and south Shetland on the moth *Amathes glareosa* Esp. A total of 7232 individuals of both forms were marked and released into the wild populations.

3. On Unst, north Shetland, where heavy predation was shown to take place by the common gull and other birds, a 7 per cent advantage of the melanic form over *f. typica* is indicated from our recaptures, though this is not significant.

4. In south Shetland where no predation was witnessed, there was no differential elimination disclosed from our recapture data.

5. An examination of the stomach contents of the common gull showed that in north Shetland, Lepidoptera, and in particular *A. glareosa*, formed one if not the chief source of food in the month of August. We have produced some evidence that they predate selectively.

6. A habit difference in which the melanic *f. edda* on Unst exhibit diminished flight activity compared to *f. typica* from south Mainland was demonstrated by three separate methods.

7. We have not yet been able to establish whether this difference in flight activity is associated with the *f. edda* or with the north and south populations.

8. In north Shetland and possibly Fair Isle, the wild type *A. glareosa* emerge later in the season than *f. edda*. Possible causes of this are discussed.

9. This polymorphism is almost certainly of considerable antiquity. It is maintained by a balance of colour, behavioural (and probably also physiological) differences. In the north efficient camouflage and diminished flight activity outweigh the advantages of the normal disruptive pattern and increased dispersal which are favoured in south Shetland as elsewhere in Britain.

FIG. 1.—Stomach contents taken from two common gulls shot on Unst, 1960.

FIG. 2.—Common gull regurgitating *A. glareosa*, Unst 1960.

Addendum

This present work is being undertaken with the object of finding out the uses of melanism in the past. This might throw light on the origin of the present-day industrial melanics. I therefore decided to introduce a number of *f. edda* ova from Shetland of known gene-frequency into a circumscribed industrial area and to see if they subsequently behaved as industrial melanics. The oasis of Sutton Park, Birmingham, seemed ideally situated for this. *A. glareaosa f. typica* is common here but no melanic forms occur. On 12th September 1960 we distributed 10,000 (± 500) ova with a gene-frequency of approximately 84 per cent. *f. edda* onto the heather slopes of the Park. Due to the dark colouration of the ground it is likely that *f. edda* may have a cryptic advantage; theoretically also diminished flight activity may contain it within the bounds of this limited area. The analogy of Sutton Park to an island is not altogether misplaced. It will be of interest to see whether in the future *f. edda* survives and behaves as an industrial melanic in this area.

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