

Temporal variation in elytral colour polymorphism in *Hippodamia variegata* (Coleoptera: Coccinellidae)

ALOIS HONEK¹, ZDENKA MARTINKOVA¹, PAVEL SASKA¹ and ANTHONY F.G. DIXON²

¹Crop Research Institute, Drnovská 507, 16106 Praha 6 – Ružyně, Czech Republic, e-mails: honek@vurv.cz; martinkova@vurv.cz; saska@vurv.cz

²University of East Anglia, School of Biological Sciences, Norwich NR4 7TJ, United Kingdom, e-mail: a.f.dixon@uea.ac.uk

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Abstract. Lady beetles (Coleoptera: Coccinellidae) are a classical group for studying the mechanisms that determine local and temporal trends in colour polymorphism. Here we report long term trends in variation in the percentage of different morphs in a population of *Hippodamia variegata* (Goeze) at Štúrovo, Slovakia (47°48'N, 18°43'E). The morphs differ in the number and location of the spots on their elytra. Beetles were sampled from stands of herbaceous plants using a standard method each year in August over a period of 74 years from 1937 to 2011. Twenty two morphs (out of 74 possible) were recorded in a total sample of 6,984 individuals. Four dominant morphs made up 90% of the total sample and varied in their annual frequency independently of one another. Frequency of “pale” morphs (0–3 spots per elytra), supposedly favoured by a warm climate, increased from 1981 to 2000s' during a period of climate warming, but only after a decrease that took place between 1937 and 1981, which did not parallel a change in climate. Moreover, the differences in the extent of the melanization of the elytral surface are too small to significantly affect thermoregulation in the different morphs. Therefore, the results presented do not provide unequivocal support for climate change determining the long term trends in the variation in the proportions of the different morphs.

INTRODUCTION

Morphological polymorphisms are well studied and provide evidence of natural variation and micro-evolutionary processes occurring in nature (Ford, 1964). Although these studies now seem “marginal” compared to the flood of information from molecular studies (Lowe et al., 2004) they are important because they provide direct evidence of interaction between environmental factors and animal characteristics at the organismic level. Among the frequently studied polymorphic taxa lady beetles (Coleoptera: Coccinellidae) occupy a prominent position, in particular the results of studies on the polymorphism in the pattern and colour of their dorsal surface: head (Rogers et al., 1971), scutum (Blehnman, 2007) and elytra. The pattern on the elytra varies in terms of the base colour and number, size and shape of spots. The few species that are the classical subjects of these studies have a small number of morphs in which the base colour of the elytra differs and is determined by major genes. There are many studies on local and temporal variation in proportions of morphs in populations (Sloggett & Honek, 2012). In most cases the occurrence of morphs appears to be associated with climatic factors, industrial pollution (Zakharov, 2003) and, possibly, visual predation (Brakefield, 1985). It is supposed that “melanic” morphs have an advantage over “pale” non-melanic morphs in cold and/or humid areas. Dark coloured individuals are able to quickly increase their body temperature when sunlight penetrates overcast skies and the resultant greater activity results in an increase in breeding success (Brakefield & Willmer, 1985; de Jong et

al., 1996). However, mechanisms balancing the ratio of particular morphs in populations are still open to dispute and worthy of further study (Majerus, 1994).

Of particular interest is the maintenance of polymorphism in species that do not show conspicuous variation in the base colour of their elytra, which is supposed to be important for the operation of selection in these species. An example is *Hippodamia* (= *Adonia*) *variegata* (Goeze), a species in which the morphs differ only in the number, size and merging of the spots. This Palearctic species is found also in central Africa and India (Bielawski, 1959) and was introduced into North (Schaefer & Dysart, 1988) and South America (Grez et al., 2004). In central Europe this species colonizes stands of wild herbaceous plants and crops infested with a variety of aphid species (Honek, 1985; Nedved, 1999). As this species has 2–3 generations per year, all of which may overwinter, this species is abundant in late summer. There is a very rich variety of morphs in this species, which differ in the pattern of spots on their elytra. The proportion of the different morphs in local populations varies geographically. In the Western Palearctic the dominant morphs lack confluent spots but in the Eastern Palearctic the dominant morphs are “melanic” with confluent spots. It has been concluded that the proportion of “melanic” morphs in an area increases with the level of humidity recorded there (Dobzhansky, 1933). At localities in Central Europe the proportions of the different morphs in local populations are similar (Schilder, 1952). The population of this species at Štúrovo, southern Slovakia, provides an excellent opportunity to study the temporal changes in the percentages of the various morphs as it has been sampled repeat-

edly since the 1930's, by Strouhal (1939), Balthasarova-Hrubantova (1951) and the present authors. To explain this temporal variation, we proposed the hypothesis that the variation in occurrence of beetles with larger or smaller numbers of spots is correlated with climatic changes and tested for a trend in the percentage of particular morphs, with an increase in pale morphs with increase in ambient temperature expected.

MATERIAL AND METHODS

Species studied

The colour pattern on the scutum and elytra of *H. variegata* is very variable (Strouhal, 1939). The pattern on the elytra, which is the topic addressed in this study, is that of the spots located at 6 specific positions (Fig. 1). Any of these spots may be either present or absent, regardless of the state of the other spots. Thus, the pattern on each elytron may consist of 0 to 6 spots in one of 74 combinations of which 22 were recorded in this study (Fig. 1). The seventh spot, which is in the centre of the scutellum and present in all individuals, was not included in this study. Rare individuals (0.14% of the total sample) in which pairs of spots merge were classified as if the spots had not merged. In addition, the morphs were also arbitrarily classified as "pale" if there were 0–3 spots and "melanic" if there were 4–6 spots on each elytron.

Sampling

The beetles were sampled within 20 km of Štúrovo, Slovakia (47°48'N, 18°43'E, 110 m a.s.l.). The terrain in this area is flat and consists of intensely cultivated large fields bordered by woody hedgerows and interspersed with small vineyards, uncultivated steppe and broadleaved forests. Typical crops are cereals, maize, alfalfa and sugar beet. Since 1971, samples of *H. variegata* were collected by the senior author (AH), each year between 10th to 30th of August. They were sampled from stands of alfalfa and wild herbaceous plants (particularly umbellifers), using an entomological sweep net, on warm still days between 08:00–18:00 h, when adults are active in the upper stratum of vegetation. The beetles sampled were put in alcohol and later sorted in the laboratory based on their elytral pattern. Beetles collected after 2004 were preserved in alcohol. Data for 1937 (Strouhal, 1939) and 1950 (Balthasarova-Hrubantova, 1951) were retrieved from literature. The period of time covered by the data is thus 74 years.

Data processing

The beetles were each assigned to one of 7 categories, each defined by the number of spots present on the elytra (0 to 6 spots) regardless of their position and to 22 morphs each characterized by a specific number and position of the spots on the elytra (Fig. 1). The frequencies of particular morphs differed in each of the 14 years sampled. Data for 2003 and 2006 were excluded from further analysis because of small sample size. Differences in morph frequencies between other years (sample size 154 – 2,296 individuals) were tested using GL saturated model with Poisson error structure and log link function, using individual counts as a response variable and morph, year and their interaction as explanatory terms. Then particular years were grouped based on the percentages of the different morphs using cluster analysis with Euclidean distances and complete linkage, in order to remove the influence of the variation in annual sample size. Percentage frequencies of the abundant morphs (making up > 5 percent of total catch) were further tested using a z-test (Systat Software, 2006). Percentage of a

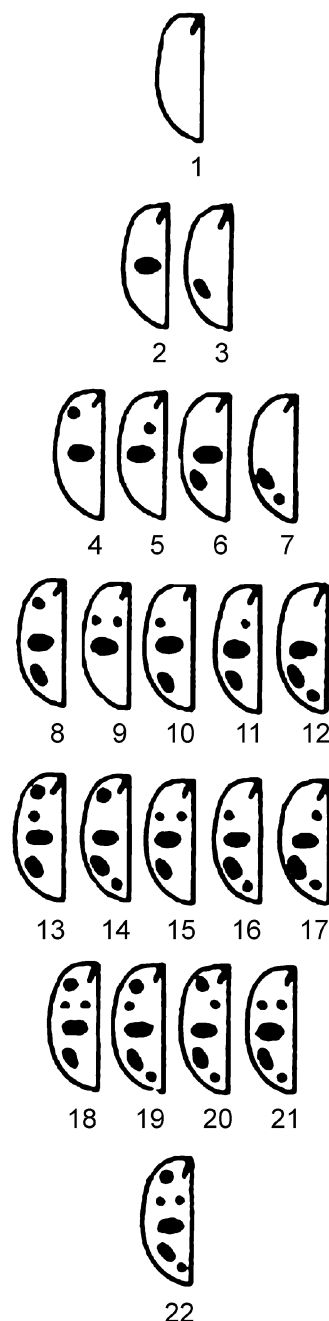


Fig. 1. Elytral colour patterns recorded during the course of this study. Numbering of the morphs throughout the text corresponds to that used in this Figure. The rows represent categories with particular numbers of spots, starting from 0 (top) to 6 (bottom).

morph in the total sample for a particular year was tested against percentage of the same morph in each of the other years.

The relationship between temperature in spring and summer and the percentage of the different morphs was investigated using data for 1992–2011. Percentages of individuals belonging to each of the categories characterised by a particular number of spots were separately regressed on average temperatures over periods (April–July, April–August, May–July and May–August) that might have affected individuals collected in late August in each of these years. This data was calculated using monthly mean temperatures (whole day temperatures calculated using standard method and data collected at 07:00 h, 14:00 h and

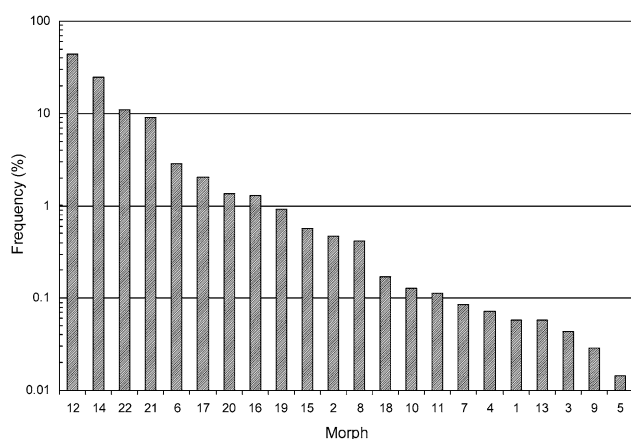


Fig. 2. Frequency of particular morphs in the total sample collected over the period 1937–2011. The morphs are indicated by the numbers used in Fig. 1.

21:00 h) at Bratislava (located c. 130 km from the sites sampled, <http://www.weatheronline.cz>). Long term records of the changes in temperature in Central Europe were obtained from Prague Klementinum, where the record goes back to 1775 (<http://zmeny-klima.ic.cz/>).

To estimate the percentage of the surface area of the elytra covered by black spots their areas were measured using an optical planimeter and photographs of individuals with spots of different sizes.

RESULTS

A total of 6,984 individuals belonging to 22 colour morphs were collected over the 74 year period from 1937 to 2011 (Appendix), which is only a fraction (30%) of the total of 74 possible morphs. Four morphs 12 (3-spot), 14 (4-spot), 21 (5-spot), 22 (6-spot) (Fig. 2), each making up > 5% of the total sample, were sampled every year and together made up 89.3% of the total sample. Four less abundant morphs, 6, 16, 17 and 20, each making up 1–5% of the total sample occurred regularly each year except 1981 (missing morph 16), 1992 and 2005 (missing morph 6), and 2010 (missing morph 20). Of the total sample they made up 7.5%. There were also fourteen rare morphs that each made up less than 1% of the total sample and together 3.2%.

The frequency of individuals with particular numbers of spots varied between years (GLM-p, deletion test: $\chi^2_{231} = 703.65$, $p < 0.001$). The percentage of “pale” morphs with 0–3 spots was high in 1937, then decreased to a minimum in 1981 and again increased up to 2006–2011 (Fig. 3). Particular years were grouped in two main clusters (Fig. 4), one consisting of 1981, 1992 and 2005, years in which there were low percentages of the 3-spot morph 12, and high percentages of 5-spot (21) and 6-spot (22) morphs. The other cluster included the remaining years with different combinations of morphs. This cluster includes three sub-lineages one of which includes 1937 and 2007–2010, which indicates that the morph composition of the *H. variegata* population at the end of the monitoring period was similar to that at the beginning. The percentage of particular abundant morphs varied independently of one another and the patterns of similarity in

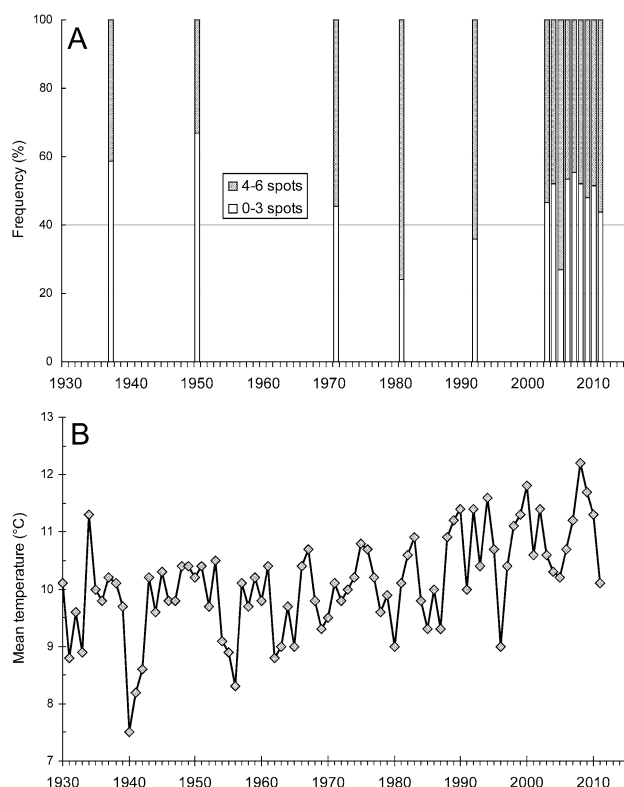


Fig. 3. A – Percentage of beetles with 0–3 spots (“pale” morphs) and 4–6 spots (“melanic” morphs) on their elytra in particular years. B – Mean annual temperatures in particular years. Data from the meteorological station of Prague Klementinum.

percentage between particular years were specific for each morph (Fig. 5).

The percentages of the abundant morphs in 1992–2011 were not significantly related to weather prevailing in the season when the beetles developed. The regression of the percentage of particular abundant morphs, 12 (3-spot), 14 (4-spot), 21 (5-spot) and 22 (6-spot), or of 3-spot plus 4-spot morphs (12 + 14) or 5-spot plus 6-spot morphs (21 + 22) on any combination of April to August mean tem-

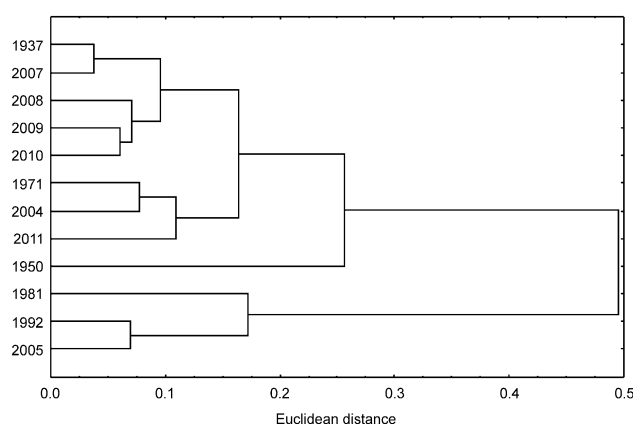


Fig. 4. Results for the years sampled grouped according to the frequency of individuals with different numbers of spots in the total sample using cluster analysis (complete linkage, Euclidean distance).

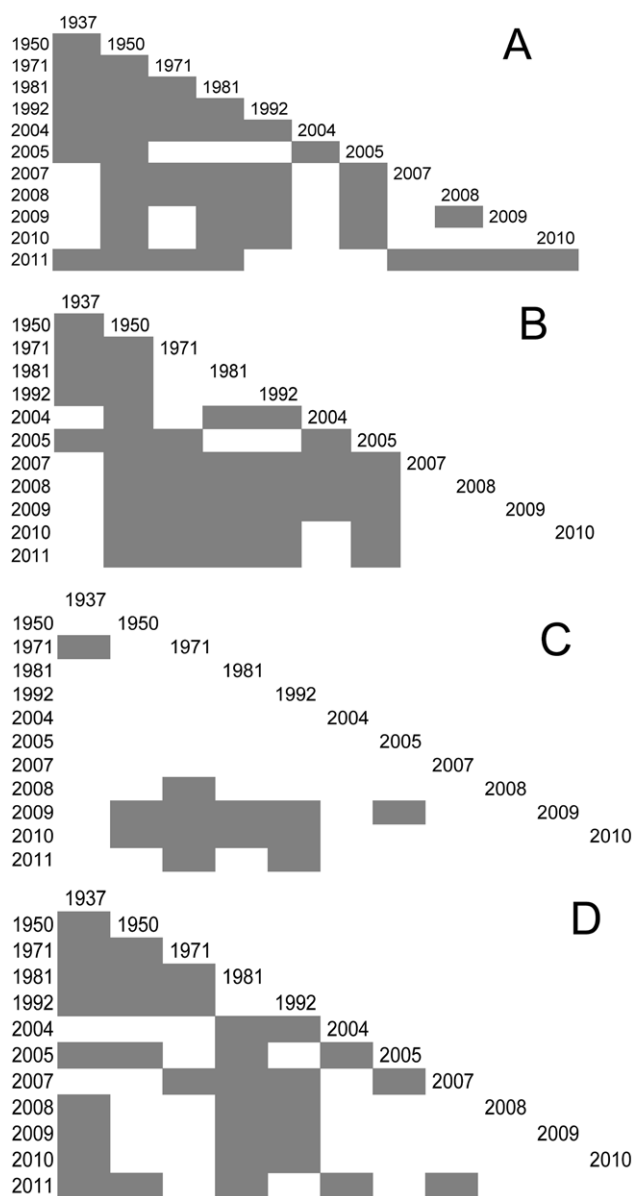


Fig. 5. Significant differences between particular years ($P < 0.05$, z-test of percentages, shaded) in percentages of abundant morphs (numbering as in Fig. 1). A – 3-spot morph 12; B – 4-spot morph 14; C – 5-spot morph 21; D – 6-spot morph 22.

perature were not significant ($P = 0.171\text{--}0.540$). In fact, the morphs were similar in the percentage of the surface of their elytra covered by black spots, which was 9% (when the spots were small) – 15% (spots large) in 3-spot morph 12, 10–19% in 4-spot morph 14, 11–19% in 5-spot morph 21 and 12–22% in 6-spot morph 22.

DISCUSSION

First, we should address a problem associated with this type of study, which is the absence of a genetic analysis of spot pattern polymorphism in *H. variegata* (Majerus, 1994). In coccinellids where the morphs differ fundamentally in pattern and base coloration of the elytra, the inheritance the pattern is via multiple alleles of a major gene. This is the case in *Adalia bipunctata* (L.) (Schröder,

1909; Fiori, 1928; Lusi, 1928), *Harmonia axyridis* (Pallas) (Tan & Li, 1934; Hosino, 1936) and *Olla v-nigrum* (Mulsant) (Vandenberg, 1992) in which melanic morphs are dominant and in *Coelophora* species (Chazeau, 1980; Houston & Hales, 1980) in which the pale morph is dominant. *Hippodamia variegata* only varies in the pattern of the distribution of the spots on the elytra. Of 74 possible spot combinations only 22 (30%) were present in the natural populations studied, which indicates there may be a genetic constraint on the extent of the variation. In other coccinellid species similar variation, although under genetic control (Lusi, 1932; Zarapkin, 1938; Hosino, 1942), is modified by the temperature experienced by an insect at certain sensitive stages in its development (Zimmermann, 1931; Okuda et al., 1997; Michie et al., 2010). With increasing temperature the size of spots decreases gradually until some spots do not develop at high temperatures. In *H. variegata* the variation in the size of spots is constrained with the lower limit to their size usually far from zero. The assumed reduction in size of spots caused by experiencing high temperatures during development probably rarely ends in spot elimination. The pattern of spots is likely to be determined genetically but this needs to be verified.

The results provide an opportunity to discuss the temporal variation in the percentage of individuals with different elytral patterns in *H. variegata*. As the data contradicted the hypothesis of stable morph composition we first looked for variation consistent with change in climatic factors already demonstrated in other coccinellid species. The composition of morphs in populations of *A. bipunctata* in xeric Central Europe has remained stable for 33 years (Honek et al., 2005) while in populations in areas with a humid oceanic climate it has changed. It is suggested that these changes are determined by the level of industrial air pollution (Creed, 1971; Zakharov, 2003) and changes in climate (de Jong & Brakefield, 1998; Brakefield & de Jong, 2011). Directional long-term changes also occur in local populations of *H. axyridis* (Komai & Chino, 1969). In both these species the temporal variation in the frequency of morphs involves changes in the percentages of “pale” (yellow or red background colour with black spots) and “melanic” (black with yellow or red spots) morphs. In contrast, in *H. variegata* there are several morphs only arbitrarily divided into “pale” and “melanic” forms. If we assume that “melanic” morphs (4–6 spots) are at an advantage when the weather is cold and overcast and the “pale” morphs (0–3 spots) when the weather is warm, then the long term variation in percentages of different morphs should parallel climate trends, but the results indicate they only partly parallel these trends (Fig. 3). The temperatures remained low between 1930 and 1980 then gradually increased up to the end of the period studied (Anonymous, 2010). During the latter period the highest increase in temperature was for April–August and decrease in rainfall for April–June, which was accompanied by an increase in the number of hours of sunshine (Anonymous, 2010). In fact changes in percentages of the different morphs of *H. variegata* fitted

the prediction after 1980 when the percentage of “pale” morphs increased in parallel with the warming in the weather. However, the decrease in the percentage of “pale” morphs between 1937 and 1981 is not paralleled by a cooling in the climate. Another reservation, when seeking an explanation for changes in the percentage composition of the different morphs in terms of climatic change, is the existence of short term oscillations in morph composition (2005), which might bias the estimation of long term trends when using incomplete data.

Studies on species with morphs that differ in the base colour of their elytra and pattern of spots reveal the crucial role of thermoregulation in balancing morph proportions (Sloggett & Honek, 2012). They lead to local differences in the proportions of the different morphs (Komai & Hosino, 1951; Bengtson & Hagen, 1975) and can change relatively fast when the climate changes (Brakefield & Lees, 1987; de Jong & Brakefield, 1998; Majerus & Zakharov, 2000; Brakefield & de Jong, 2011). It is unlikely that thermoregulation has affected the percentages of the different morphs in the Sturovo population of *H. variegata*. The differences in the median percentages of the surface area of the elytra covered with melanic spots in the most abundant morphs are small and overlapping. Therefore the variation in percentage of elytral surface that is melanic is unlikely to result in significant differences in the body temperatures of the different morphs.

Temporal changes are important when evaluating local variation in the percentages of different morphs. The composition of the population at Sturovo is generally similar to that of populations at other localities in Central (Schilder, 1928, 1952; Schilder & Schilder, 1951) and Eastern Europe (Dobzhansky, 1933). The occurrence of particular morphs is nearly identical [one morph lacking confluent spots in Schilder’s (1952) list was not present in our samples] and the order of abundant morphs is similar. The major difference is in the variation in the percentages of the rarer morphs. As there is a significant temporal variation in morph composition of local populations, geographic comparisons based on data collected in different years are confounded by temporal variation in percentages of the different morphs.

Causes of the significant long term variation in percentages of the different morphs of *H. variegata* thus remain to be determined. As in other species further progress requires an experimental study of the genetic basis of colour polymorphism and the reaction norms of the various morphs to temperature. This supplementary information will make this species a likely subject for studies on ecological genetics.

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APPENDIX 1. Numbers of individuals of morphs 1–22 (Fig. 1) in samples collected in years 1937–2011.

Morph	1937	1950	1971	1981	1992	2003	2004	2005	2006	2007	2008	2009	2010	2011	Total
1	0	1	1	0	0	0	0	0	0	0	0	0	0	2	4
2	3	4	11	1	1	2	0	2	0	1	3	3	1	1	33
3	0	2	1	0	0	0	0	0	0	0	0	0	0	0	3
4	2	1	0	1	0	0	0	1	0	0	0	0	0	0	5
5	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
6	25	45	62	2	0	0	9	5	0	11	10	11	8	12	200
7	1	0	2	0	0	0	1	0	0	0	0	2	0	0	6
8	8	1	9	0	0	0	1	0	0	1	2	2	0	5	29
9	0	1	1	0	0	0	0	0	0	0	0	0	0	0	2
10	1	1	1	0	0	0	3	0	0	0	0	1	0	2	9
11	1	2	1	0	0	0	1	0	0	1	0	1	0	1	8
12	255	461	957	32	112	18	114	114	8	138	288	310	123	189	3119
13	0	0	1	0	0	0	0	2	0	0	0	0	1	0	4
14	98	97	699	59	108	11	64	136	2	50	102	133	57	112	1728
15	4	9	8	0	0	0	1	2	0	3	2	1	1	9	40
16	13	6	28	0	5	1	2	2	1	13	3	7	2	6	89
17	9	8	40	4	6	0	6	4	2	7	19	26	2	9	142
18	0	0	6	1	0	0	0	0	0	0	2	2	0	1	12
19	1	5	24	5	2	1	3	7	0	0	1	14	0	1	64
20	5	3	24	2	6	0	5	11	0	5	10	12	0	11	94
21	53	66	164	9	20	3	20	32	0	26	59	89	34	55	630
22	25	63	256	37	54	7	18	54	2	18	60	73	27	68	762
Total	504	776	2296	154	314	43	248	372	15	274	561	687	256	484	6984