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ORIGINAL RESEARCH ARTICLE

Results of international standardised beekeeper surveys of colony losses for winter 2012-2013: analysis of winter loss rates and mixed effects modelling of risk factors for winter loss

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Summary

This article presents results of an analysis of winter losses of honey bee colonies from 19 mainly European countries, most of which implemented the standardised 2013 COLOSS questionnaire. Generalised linear mixed effects models (GLMMs) were used to investigate the effects of several factors on the risk of colony loss, including different treatments for *Varroa destructor*, allowing for random effects of beekeeper and region. Both winter and summer treatments were considered, and the most common combinations of treatment and timing were used to define treatment factor levels. Overall and within country colony loss rates are presented. Significant factors in the model were found to be: percentage of young queens in the colonies before winter, extent of queen problems in summer, treatment of the varroa mite, and access by foraging honey bees to oilseed rape and maize. Spatial variation at the beekeeper level is shown across geographical regions using random effects from the fitted models, both before and after allowing for the effect of the significant terms in the model. This spatial variation is considerable.

Modelización de efectos mixtos en los factores de riesgo de la pérdida de colonias de invierno en 2012-13 según las encuestas estandarizadas internacionales realizadas a los apicultores

Resumen

Este artículo presenta los resultados de un análisis de los datos de la tasa de pérdida de invierno de 19 países, en los que se aplicó mayoritariamente el cuestionario estandarizado Coloss 2013. Se usaron modelos de efectos mixtos generalizados lineales para investigar los efectos de varios factores sobre el riesgo de pérdida de colonias de abejas de la miel, incluyendo diferentes tratamientos para *Varroa destructor*, y teniendo en cuenta los efectos aleatorios del apicultor y la región. Se consideraron tanto los tratamientos de invierno como los de verano, y las combinaciones más comunes de tratamiento y momento del mismo para definir los niveles del factor tratamiento. En general y dentro de cada país se presentaron pérdidas proporcionales. Los factores significativos del modelo fueron: porcentaje de reinas jóvenes en las colonias antes del invierno, alcance de los problemas de la reina en el verano, el tratamiento contra Varroa, y el acceso de las abejas pecoreadoras a las semillas oleaginosas y el maíz. La variación espacial al nivel de apicultor se muestra en todas las regiones geográficas utilizando los residuos del modelo ajustado final, después de considerar el efecto de los términos significativos en el modelo. Existe una considerable variación espacial.

Keywords: honey bee colony losses, generalised linear mixed modelling (GLMM), random effects, *Varroa destructor,* beekeeper variation*,* regional variation, questionnaire data, COLOSS

Introduction

High honey bee colony losses have been observed in recent years in many countries (Neumann and Carreck, 2010; Potts *et al.*, 2010), notably from 2006 onwards in the USA (Ellis *et al.*, 2010; vanEngelsdorp *et al.*, 2007, 2008, 2010, 2011, 2012; Spleen *et al.*, 2013; Steinhauer *et al.*, 2014) but subsequently in many other places (Aston, 2010; Brodschneider *et al.*, 2010; Charriere and Neumann, 2010; Currie *et al.*, 2010; Gray *et al.*, 2010; Hatjina *et al.*, 2010; Ivanova and Petrov, 2010; Mutinelli *et al.*, 2010; Tlak Gajger *et al.*, 2010; Topolska *et al.*, 2010; Vejsnæs *et al.*, 2010; van der Zee, 2010; van der Zee *et al.*, 2012; Clermont *et al.*, 2014; Pirk *et al.*, 2014). This has led to intensive co-operation between honey bee experts to investigate this problem from different perspectives, including epidemiology and experimental approaches. A milestone in this co-operation was the formation in 2008 of the honey bee research network COLOSS (Prevention of honey bee COlony LOSSes; www.coloss.org), intended to intensify contacts and research collaboration between honey bee experts (Neumann and Carreck, 2010). The activities of the COLOSS working group for ‘Monitoring and Diagnosis’ (Nguyen *et al.*, 2010) resulted in the production of annual internationally standardised questionnaires and the development of specific protocols to collect information from bee-keepers by means of questionnaires (van der Zee *et al.*, 2013). The aim was to collect representative information, comparable across different countries, about variation in beekeeping management practices and colony losses and hence to investigate potential risk factors.

One of the known factors contributing to colony losses is the widespread presence of the ectoparasitic mite (*Varroa destructor*) (Rosenkranz *et al.*, 2010; Le Conte *et al.*, 2010, Genersch, 2010), referred to in this article as the varroa mite. In the present study we report colony losses over winter 2012-2013 in a substantial number of European countries and Israel, and analyse, using statistical model fitting, to what extent the manner of treatment of the varroa mite and some other factors are associated with the observed losses.

To determine the effects of the different methods of varroa treatment, we investigated whether we could compose groups that represented the product and the period when it was used. Varroa treatment is mainly performed in summer (defined here as July to September) and winter (defined here as October to January) although some beekeepers do start in spring and some control methods such as drone brood trapping can be practised throughout the active season. Early treatment is mainly performed by removing drone brood, because drone brood offers the best opportunities for mite reproduction, owing to the longer period for which the drone brood is sealed compared to worker brood. In late summer, colonies produce their winter honey bee population, which must be able to survive an often long winter. These honey bees are specifically adapted to survive the long winter months (Fluri *et al.*, 1982; van Dooremalen *et al.*, 2012), but their chance of survival can be severely compromised if the larvae develop under the pressure of a high level of varroa mite infestation during this time (Fries *et al.*, 1994; Amdam *et al.*, 2004). In most European countries no or only a small amount of brood is present during the cold months of winter. Consequently (nearly) all mites are phoretic during these winter months, which makes them vulnerable to control products. For these reasons both treatment in summer and treatment in winter should be included in the final varroa treatment factor for the model. There is also a wide choice of control products available for beekeepers (Rosenkranz *et al.*, 2010). Combining every product with the two time periods would lead to many levels of the treatment factor, and hence have a large negative impact on the statistical validity of the estimated effects in the model because of over-specification. For the same reason we left out drone brood removal in spring for this analysis. The resulting treatment factor is described further in the next section.

We also report on the effect of observed problems with queens in the summer of 2012, the percentage of colonies before winter with a young queen, and environmental effects as indicated by access of foraging honey bees on maize and oilseed rape, which, owing to their frequent treatment with neonicotinoid pesticides, have been suggested as potentially harmful to honey bees (European Food Safety Authority, 2013; see also the discussion below).

An important aim of this study is presentation of the remaining unexplained losses attributable to other factors which cannot be explained by the factors identified in the questionnaire, but can be characterised as regional or local impacts on honey bee over-wintering.

Material and methods

Data collection

The data used here result from the annual return of data from the COLOSS (Neumann and Carreck, 2010) loss monitoring questionnaire (van der Zee *et al.*, 2013) for the winter of 2012-2013.The data collection approaches differed between participating countries and included census models, self-selected samples and randomly selected samples (see Table 1 in van der Zee *et al.*, 2012; van der Zee *et al.*, 2013; Gray and Peterson, 2013). All questionnaires included the same core questions considered as "essential" (see Fig. 2 in van der Zee *et al.*, 2013). Participating beekeepers in each country returned their completed surveys to their national co-ordinators, who were subsequently responsible for submitting relevant data in a standardised format to the international database by 14 July 2013 for analysis. It is necessary for practical reasons to provide a deadline for return of data each year when dealing with so many different countries. In fact no other countries returned data after that date and we have used all of the valid data available to us for the analysis. The complete questionnaire is available as supplementary material at: http://www.ibra.org.uk/downloads/20140220/download

Data were excluded from the loss rate analysis if the essential questions about colony losses were not answered or seemed to be incorrect (i.e. if more colonies were stated to have been lost than were wintered; and a few beekeepers with no colonies going into winter). Data were excluded from the analysis of varroa treatment if essential questions concerning treatment of the varroa mite were not answered. For the model fitting, only beekeepers who provided both valid loss data and responses to all of the questions concerning the variables used in the model were included. We give details in the appropriate results section of how many responses were excluded and why.

Estimation of loss rates and statistical modelling

Estimation of the overall proportion of colonies lost and overall proportion of weak colonies after winter was done using an intercept-only quasi-binomial generalised linear model (GLM) with logit link (as in van der Zee *et al.*, 2013). These estimated proportions are respectively the total number of stated colonies lost as a proportion of colonies kept at the start of the winter of 2012-2013 and the total number of weak colonies after winter 2012-2013 as a proportion of those kept at the start of winter. This is the "overall loss rate" recommended in van der Zee *et al.* (2013). It is similar to the "total loss" referred to in vanEngelsdorp *et al.* (2013a), but they take account of managed increases and decreases in colony numbers during two fixed time frames every half year in calculating the number of lost colonies. In the present study winter is used as the time frame and is defined as the period between the moment that a beekeeper finished pre-winter preparations for his/her colonies and the start of the new foraging season (as defined in van der Zee *et al.*, 2013). Most of the countries in our study are far north in the northern hemisphere and colony splitting in winter is not practised. There are a few countries included where this is done or may be done, however the number of beekeepers represented in the data from these countries is small. It is also our experience that asking questions about managed increases and decreases leads to a large proportion of the data being invalid (van der Zee *et al.*, 2012). For these reasons we did not ask for this information in the 2013 questionnaire.

Fitting the quasi-binomial GLM allows the calculation of a confidence interval for the overall loss rate taking account of extra (extra-binomial) variation in the data caused by lack of independence of colonies within beekeeper operations, i.e. a beekeeper effect.

Corresponding loss rates per country were calculated using the same approach but using country as a factor in the model (see van der Zee *et al.*, 2013, for an example of this). The loss rates per country are reported for three different sizes of operation: a maximum of 50 colonies (the largest class), 51 to 150 colonies, and more than 150 colonies.

Further model fitting was done using mixed effect regression models (also known as multilevel models), which incorporate both fixed and random effects (Zuur *et al.*, 2009; Twisk, 2010) and which allow for the nature of the data, i.e. colonies belonging to beekeepers within regions. Beekeeper and region were included as random effects, to allow for differences between beekeepers and/or regions, in a generalised linear mixed model (GLMM) using a binomial distribution. Varroa treatment, queen problems in 2012, extent of queen replacement with young queens (a covariate), migration, operation size, type of winter feed, foraging on oilseed rape or maize, and brood comb renewal were considered as fixed effects. This GLMM specifies the log-odds of winter loss as a linear function of the covariates and factors of interest (the fixed effects) and the random effects which modify the intercept (the baseline log-odds) in this linear function for different

Table 1. Extent of use of varroa treatments and corresponding overall loss rates, for 1-50 colony operations; columns 2 and 3 relate to operations which provided usable information about varroa mite treatment and columns 4 and 5 are for operations providing all relevant information for model fitting. *Not used in the final model.

	Single factor exploration		**Included in best explaining model**	
Varroa treatment 2012-13	**No. Beekeepers (%)**	**% loss rate**	**No. beekeepers (%)**	**% loss rate**
Only spring (April-June) treatment*	87 (1.0)	23.5		
Only drone brood removal*	117 (1.3)	19.0		
Only trapping comb method*	39 (0.4)	10.4		
Apistan® in summer	218 (2.5)	16.3	188 (3.3)	16.1
Amitraz in summer	116 (1.3)	20.0	48 (0.8)	21.9
Thymol in summer	512 (5.8)	23.2	389 (6.9)	22.5
Oxalic acid in summer	152 (1.7)	21.6	141 (2.5)	22.1
Formic acid in summer	728 (8.3)	21.0	547 (9.7)	20.6
Formic acid in summer + oxalic acid in winter	2690 (30.5)	16.1	1547 (27.4)	18.3
Thymol in summer + oxalic acid in winter	604 (6.8)	17.5	302 (5.3)	17.1
Formic acid + thymol in summer + oxalic acid in winter	130 (1.5)	15.3	100 (1.8)	15.1
Amitraz in summer + winter	125 (1.4)	22.0	36 (0.6)	14.5
Apistan® in summer + winter	132 (1.5)	15.8	106 (1.9)	14.5
Only winter oxalic acid/Amitraz/Apistan®	1623 (18.4)	20.5	1336 (23.6)	20.9
Only winter thymol or formic acid	145 (1.6)	32.1	118 (2.1)	29.0
Other products	646 (7.3)	22.0	475 (8.4)	22.2
Treated but no info about product*	370 (4.2)	17.9		
No treatment	389 (4.4)	23.6	319 (5.6)	23.4
Total	8823 (100)	19.0	5652 (100)	19.7

beekeepers or regions or both. The effect of country is not explicitly considered in the model, other than through the region within the country. An advantage of including the random effects in the model is that they help to explain variation in the data and therefore to reduce the standard errors of the estimated fixed effects. This means that fixed effects that are important are more likely to be statistically significant.

We only fitted random intercepts models. Random effects models can also include random slopes, which in this case would modify the effect of specified covariates or factors for different beekeepers or regions. As there is only one observation available per beekeeper in this dataset it was not possible to use random slopes at beekeeper level. It would be possible in principle to fit random slopes at regional level but we did not do this in this analysis.

Plotting these random effects in spatial maps enables us to visualise spatial characteristics of the variation in the log-odds of loss. We plotted a choropleth map (Pfeiffer *et al.*, 2008) of the region level effects and spatially smoothed maps of the beekeeper level effects (using the method of Bornmann and Waltman, 2011). The beekeeper level maps used beekeeper location if that was available, failing which apiary location was used.

The analysis was carried out in R (R Development Core Team, 2011), using library "lme4" (Bates *et al.*, 2011) and maps were produced with QGIS version 2.0.1-Dufour (available at http://www.qgis.org/en/site/index.html). The glmer function in the lme4 library was used to fit the mixed models. This uses Laplace approximation to estimate the model parameters. The quasi-binomial GLM calculations used the glm function in the "stats" library.

Determining levels of the fixed factor varroa treatment

Based on investigation of the time of varroa treatment and the product used, we created 14 treatment groups describing the combination of product and summer or winter treatment, using the most common combinations in the data in which varroa treatment information was available. Those responding "don't know/not applicable" to whether or not they treated, an option which was available only in Sweden, were excluded. A treatment was defined as performed in summer if it was started in July, August or September, and performed in winter if it was started in October, November, December or January. These treatment groups were used to define factor levels. Table 1 (columns 1 to 3) shows detailed information for beekeepers with at most 50 colonies. This group represents the largest group overall and is used for the more detailed analysis here.

Results

Response

The 19 countries returning data were, Austria, Bosnia and Herzegovina, Croatia, Denmark, Estonia, Finland, Germany, Ireland, Israel, Italy, Latvia, Lithuania, the Netherlands, Norway, Poland, Scotland, Slovakia, Sweden and Switzerland, although not all of these provided data for all of the questions analysed below. In total 15,850 beekeepers with 279,523 colonies kept at the start of winter 2012-2013 were reported to the international co-ordinator for data analysis.

Table 2. Percentage of weak colonies in spring 2013, by country, for beekeepers with 1 to 50 colonies; Croatia and Scotland did not provide this data and are omitted.

Country	**% of weak colonies in 1-50 colony operations**	**95% Confidence Interval**	**No. of beekeepers**	**No. of colonies going into winter**	**No. of weak colonies**
Austria	14.5	13.6, 15.4	854	12140	1759
Bosnia-Herzegovina	11.5	9.1, 14.4	46	1079	124
Denmark	13.8	12.8, 14.8	1184	9185	1264
Estonia	15.8	13.0, 19.0	83	1119	177
Finland	9.1	7.6, 10.9	203	2259	205
Germany	11.8	11.4, 12.2	5279	56441	6664
Ireland	24.2	21.8, 26.7	312	2316	560
Israel	28.8	20.0, 39.7	9	156	45
Italy (Veneto region)	15.5	12.0, 19.8	48	663	103
Latvia	15.4	14.3, 16.7	423	7260	1122
Lithuania	7.7	4.7, 12.2	30	404	31
Netherlands	10.7	9.9, 11.5	1568	11445	1224
Norway	15.5	14.3, 16.9	400	6018	935
Poland	14.6	13.4, 15.8	300	6651	969
Slovakia	11.8	9.9, 14.0	95	1889	222
Sweden	14.3	13.5, 15.1	1643	14014	1998
Switzerland	6.1	5.7, 6.7	1305	17165	1054

Weak colonies after winter 2012-2013

Not all countries provided information on the number of their colonies which survived the winter of 2012-2013 but in a weak condition, and not all beekeepers responded to this question in countries which did ask the question. Croatia and Scotland did not provide information on weak colonies. From the other countries, 14,514 beekeepers answered this question, of whom 14,501 provided valid data. These 14,501 beekeepers were managing 250,889 colonies before winter, of which 28,898 colonies were stated as being weak after winter. The overall proportion of weak colonies, of those wintered, was therefore 11.5%, with a 95% confidence interval of (11.3%, 11.7%) (from a quasi-binomial glm).

There were 13,782 beekeepers with 1 to 50 colonies, 581 with 51 to 150 colonies and 138 with 151 or more colonies. The 1-50 colony group wintered 150,204 colonies and had 18,456 weak colonies in spring 2013, a percentage of 12.3% with 95% confidence interval of (12.0%, 12.5%). An analysis of the proportions of weak colonies for this largest group of beekeepers by country is given in Table 2. There are highly significant differences between countries in terms of these proportions. Ireland and Israel had the highest proportions of weak colonies, at about 24% and 29% respectively, and Switzerland and Lithuania had the lowest proportions (about 6% and 7% respectively).

Winter 2012-2013 losses

In total 15,720 beekeepers providing valid loss data (out of all 15,850 respondents) wintered 277,609 colonies kept at the start of winter 2012-2013 and 44,681 colonies were lost over winter. The overall loss rate (total colonies lost as a proportion of colonies wintered; van der Zee *at al.*, 2013) was 16.1% with a 95% confidence interval of (15.8%, 16.4%) (from a quasi-binomial glm). There were 14,879 beekeepers with 1 to 50 colonies, 687 with 51 to 150 colonies and 154 with 151 or more colonies. The 1-50 colony group wintered 161,495 colonies and lost 28,409, a 17.6% loss rate with 95% confidence interval of (17.2%, 17.9%). The 51 to 150 colony group wintered 56,373 colonies and lost 9,256, a loss rate of 16.4% with 95% confidence interval (15.1%, 17.8%). The 151 or more colony group wintered 59,741 colonies and lost 7,016, a loss rate of 11.7% with a 95% confidence interval of (9.9%, 13.8%), which is a significantly lower loss rate than for the smaller beekeeping operations.

Losses were analysed at regional level through the model fitting below. Table 3 shows the numbers of beekeepers with valid loss data for each size of beekeeping operation, and the total number of such beekeepers in each country, as well as the corresponding numbers of honey bee colonies. It can be seen that in almost all cases the 1-50 colony operations account for by far the most beekeepers. Amongst these countries, only Israel has more larger scale operations than smaller scale ones. For comparison, Table 3 also shows the estimated number of beekeepers in each country in 2012. Loss rates per country are reported, by size of operation and with 95% confidence intervals, in tables 4 to 6.

There are highly significant differences between the loss rates in different countries, for each size of operation. In the 1-50 colony category, the loss rates vary from just under 10% for Slovakia and Bosnia-Herzegovina to over 36% for Scotland and nearly 39% for Ireland (see Table 4). For beekeepers with 51 to 150 colonies (see Table 5), the loss rate was again low for Bosnia-Herzegovina (about 5%) but also for Lithuania (7%), Israel (5%) and one Italian region (4%), although the numbers of beekeepers are very low for these last two

Table 3. Breakdown of beekeeper and colony numbers by size of beekeeping operation, for beekeepers with valid loss data.

	1-50 colony operations		**51-150 colony operations**		**151 or more colony operations**		**Overall sample figures**		**Estimate for whole country**
Country	**No. of beekeepers**	**No. of colonies going into winter**	**No. of beekeepers**	**No. of colonies going into winter**	**No. of beekeepers**	**No. of colonies going into winter**	**Total no. of beekeepers in sample**	**Total no. of colonies going into winter**	**Estimated total no. of beekeepers in the country, in 2012**
Austria	931	13034	54	4430	10	2030	995	19494	25099
Bosnia-Herzegovina	46	1079	25	1956	1	280	72	3315	4115
Croatia	104	2445	70	6029	7	1358	181	9832	9000
Denmark	1185	9186	30	2587	10	2681	1225	14454	4600
Estonia	84	1127	5	475	7	1773	96	3375	5934
Finland	218	2567	31	2628	11	5323	260	10518	2600
Germany	5857	60605	139	10883	9	2072	6005	73560	100000
Ireland	372	2480	11	1085	-	-	383	3565	2600
Israel	11	200	5	385	19	23078	35	23663	500
Italy (Veneto region)	52	763	2	270	-	-	54	1033	55000
Latvia	432	7356	68	5722	19	4842	519	17920	4300
Lithuania	30	404	10	766	4	1860	44	3030	9000
Netherlands	1571	11457	15	1063	3	1400	1589	13920	7000
Norway	401	6028	52	4546	20	4705	473	15279	2800
Poland	439	9274	69	5049	3	855	511	15178	51778
Scotland	98	400	1	70	-	-	99	470	1300
Slovakia	95	1889	21	1833	4	736	120	4458	16300
Sweden	1648	14036	62	5296	27	6748	1737	26080	12000
Switzerland	1305	17165	17	1300	-	-	1322	18465	16000

Table 4. Overall loss rates for winter 2012-2013 for beekeepers with 1 to 50 colonies, by country.

Country	**% loss rate in 1-50 colony operations**	**95% Confidence interval**	**No. of beekeepers used**	**No. of colonies going into winter**	**No. of colonies lost**
Austria	18.1	16.9, 19.3	931	13034	2358
Bosnia-Herzegovina	9.7	7.0, 13.4	46	1079	105
Croatia	11.1	9.1, 13.6	104	2445	272
Denmark	21.2	19.7, 22.7	1185	9186	1945
Estonia	24.4	20.1, 29.2	84	1127	275
Finland	21.3	18.5, 24.3	218	2567	546
Germany	15.2	14.7, 15.8	5857	60605	9239
Ireland	38.9	35.4, 42.4	372	2480	964
Israel	20.0	11.8, 31.9	11	200	40
Italy (Veneto region)	14.9	10.9, 20.1	52	763	114
Latvia	21.4	19.7, 23.1	432	7356	1573
Lithuania	18.8	12.8, 26.7	30	404	76
Netherlands	14.3	13.2, 15.5	1571	11457	1642
Norway	19.7	18.0, 21.6	401	6028	1189
Poland	20.9	19.4, 22.4	439	9274	1939
Scotland	36.2	28.2, 45.1	98	400	145
Slovakia	9.3	7.2, 12.0	95	1889	176
Sweden	24.3	23.0, 25.6	1648	14036	3408
Switzerland	14.0	13.1, 15.0	1305	17165	2403

Table 5. Overall loss rates for winter 2012-2013 for beekeepers with 51 to 150 colonies, by country; confidence intervals were not estimated when there were fewer than 5 respondents in the relevant group.

Country	% loss rate in 51-150 colony operations	95% Confidence Interval	No. of beekeepers	No. of colonies going into winter	No. of colonies lost
Austria	16.1	12.1, 21.2	54	4430	715
Bosnia-Herzegovina	4.9	2.1, 10.9	25	1956	96
Croatia	10.2	7.4, 13.9	70	6029	615
Denmark	16.0	10.9, 22.9	30	2587	414
Estonia	21.5	9.8, 40.8	5	475	102
Finland	16.6	11.4, 23.4	31	2628	435
Germany	16.7	13.9, 19.8	139	10883	1813
Ireland	33.0	22.4, 45.6	11	1085	358
Israel	5.2	0.8, 26.8	5	385	20
Italy (Veneto region)	4.4	-	2	270	12
Latvia	19.3	15.4, 24.0	68	5722	1105
Lithuania	7.0	2.3, 19.6	10	766	54
Netherlands	13.2	6.7, 24.3	15	1063	140
Norway	15.1	11.2, 20.0	52	4546	686
Poland	14.6	11.0, 19.2	69	5049	739
Scotland	1.4	-	1	70	1
Slovakia	9.6	5.2, 16.9	21	1833	175
Sweden	27.8	23.0, 33.1	62	5296	1471
Switzerland	23.5	15.2, 34.5	17	1300	305

Table 6. Overall loss rates for winter 2012-2013 for beekeepers with 151 colonies or more, by country; Ireland, Italy, Scotland and Switzerland had no such beekeepers in their data samples and are omitted. Confidence intervals were not estimated when there were fewer than 5 respondents in the relevant group.

Country	% loss rate in 151 plus colony operations	95% Confidence Interval	No. of beekeepers	No. of colonies going into winter	No. of colonies lost
Austria	14.3	7.5, 25.8	10	2030	291
Bosnia-Herzegovina	1.4	-	1	280	4
Croatia	3.6	0.7, 16.8	7	1358	49
Denmark	24.8	16.4, 35.6	10	2681	664
Estonia	27.2	16.8, 40.9	7	1773	482
Finland	15.2	10.3, 21.8	11	5323	809
Germany	12.4	6.2, 23.4	9	2072	257
Israel	5.8	4.2, 7.8	19	23078	1337
Latvia	17.4	12.0, 24.6	19	4842	844
Lithuania	5.4	-	4	1860	100
Netherlands	9.0	-	3	1400	126
Norway	19.0	13.3, 26.6	20	4705	896
Poland	9.6	-	3	855	82
Slovakia	7.6	-	4	736	56
Sweden	15.1	10.7, 20.9	27	6748	1019

countries. Ireland again had the highest losses (33%), though Sweden, Switzerland and Estonia suffered high losses also (about 28%, 23% and 21% respectively). Scotland only had one beekeeper in the sample with this size of operation and a very low loss rate. There are fewer countries represented in the 151 or more colonies category (see Table 6), but again the loss rates are rather variable, from under 2% for Bosnia-Herzegovina to about 25% and 27% for Denmark and Estonia respectively.

Results of model fitting

Before assessing the significance of the fixed factors for risk of loss, we fitted a null (intercept only) generalised linear mixed model for the 14,756 respondents out of the 14,879 respondents with valid loss data and at most 50 colonies who also provided information on bee-keeper or apiary location. This model included a random effect (random intercept) at beekeeper level and also at region level. Examining the random effects or random intercepts shows the deviation

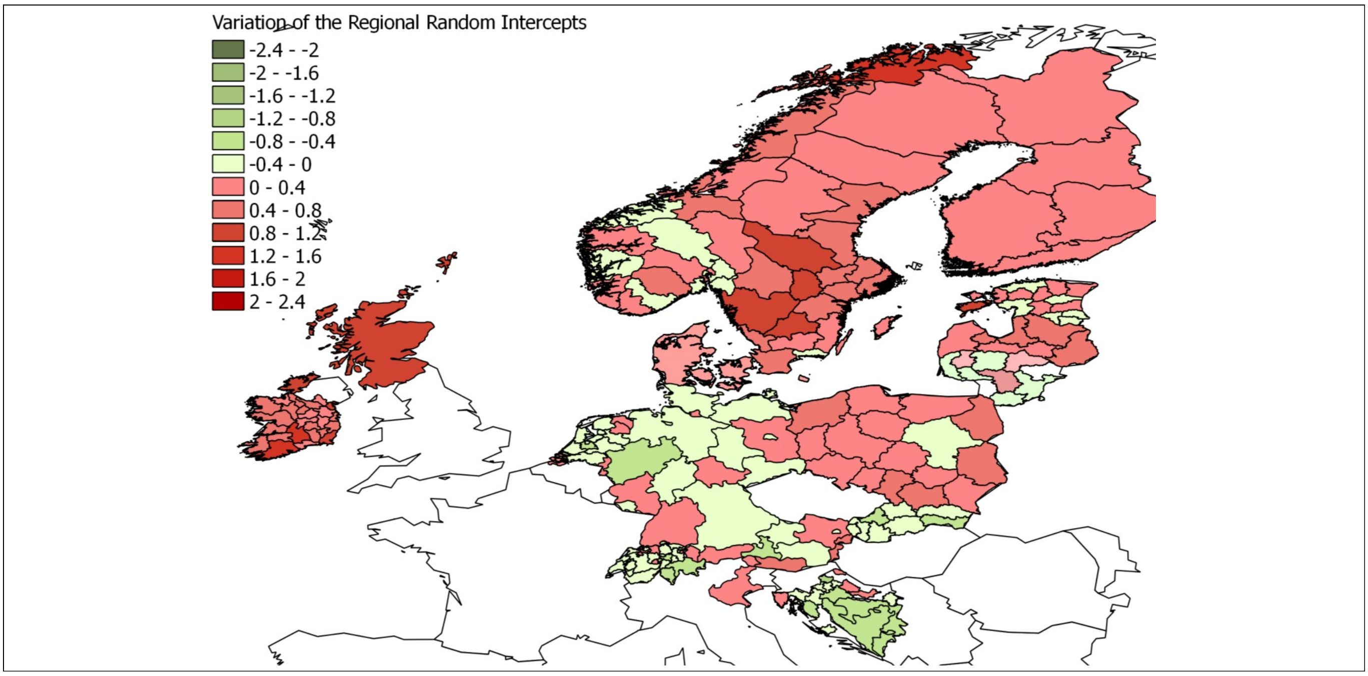

Fig. 1. Choropleth map showing the spatial variation in the region level random intercepts in a binomial GLMM without any fixed factors for beekeeper risk of colony loss in European countries, for the 1 to 50 colony operations. The legend shows the key to the colour coding of the random effects. Darker green indicates areas of lower risk and darker red areas of higher risk of loss.

or variation in the baseline log-odds of loss for the beekeeper or the region from the average baseline log-odds before allowing for the model covariate and factors.

Fig. 1 shows a choropleth map of the size of the region level random effects from the null model. These are especially high, indicating areas where risk of loss is higher (darker red parts of the map) in Scotland (treated as one region), parts of Ireland, large areas in the south of Sweden, and especially in the north of Norway. The areas of lower risk, indicated in green, are substantial parts of Austria, the Baltic states (Latvia, Lithuania and Estonia) Bosnia-Herzegovina, Croatia, Germany, the Netherlands, Slovakia and Switzerland.

Fig. 2 shows a map of the spatially smoothed beekeeper level random effects from the null model. There are many darker red areas in Fig. 2 within different countries, indicating higher risks of winter loss for beekeepers in those areas. This is especially true for Ireland, and much of Denmark and the south of Sweden, with local areas of higher risks of winter colony loss in many places elsewhere. Bosnia-Herzegovina, Croatia and Slovakia again generally have low risks of winter loss. For the further model fitting we also used beekeepers with 1 to 50 colonies. The information on the fixed factors was not available for all of these beekeepers. In total 8,823 beekeepers provided information about, at least, the combination of varroa period and treatment, while 5,652 beekeepers from 139 regions provided all of the required data. Table 1, columns 4 and 5, gives details about the extent of use of the different varroa treatments for this subpopulation, which is similar to the information in columns 2 and 3 for the larger dataset. The number of respondents omitted from the analysis for reasons relating to their responses concerning varroa treatment was 257, comprising 53 Swedish beekeepers who responded "don't know/not applicable" to whether or not they treated, 2 not responding at all, and 202 who treated but provided no further information on treatment. We included the 319 beekeepers who did not treat for varroa.

Again both beekeeper and region were used as random effects in the GLMM. Screening the data for significant explanatory factors for the risk of loss, one factor at a time, varroa treatment as above (termed "varroa" below), the percentage of new 2012 queens in the wintered colonies ("percentage_newqueen", as a covariate), queen problems in 2012 ("queenproblem2012": more than normal, normal, less than normal, don't know), access to oilseed rape as forage ("rape": yes, no, don't know) and access to maize ("maize": yes, no, don't know) were all highly significant fixed effects in the mixed model. The baseline categories used in the model for the factors were: varroa treatment = only summer treatment with formic acid, queen problems = more than normal, rape = yes, maize = yes.

A full model was constructed including all of these significant variables as well as the two random effects, and all of them remained significant when tested singly and also as a group. The estimated variances of the random effects in the final model containing all of these fixed factors were 1.52 for beekeeper and 0.22 for region, compared to 1.58 and 0.31 respectively for the same random effects in the corresponding null model. In each case the variance is larger for beekeeper than region. The variance reduces more for region than beekeeper when the fixed effects are included in the model. We also compared maps of the beekeeper level random effects in the full model and in the null model fitted using these 5,652 beekeepers only. Fig. 3 shows the map for the full model. The map for the null model was

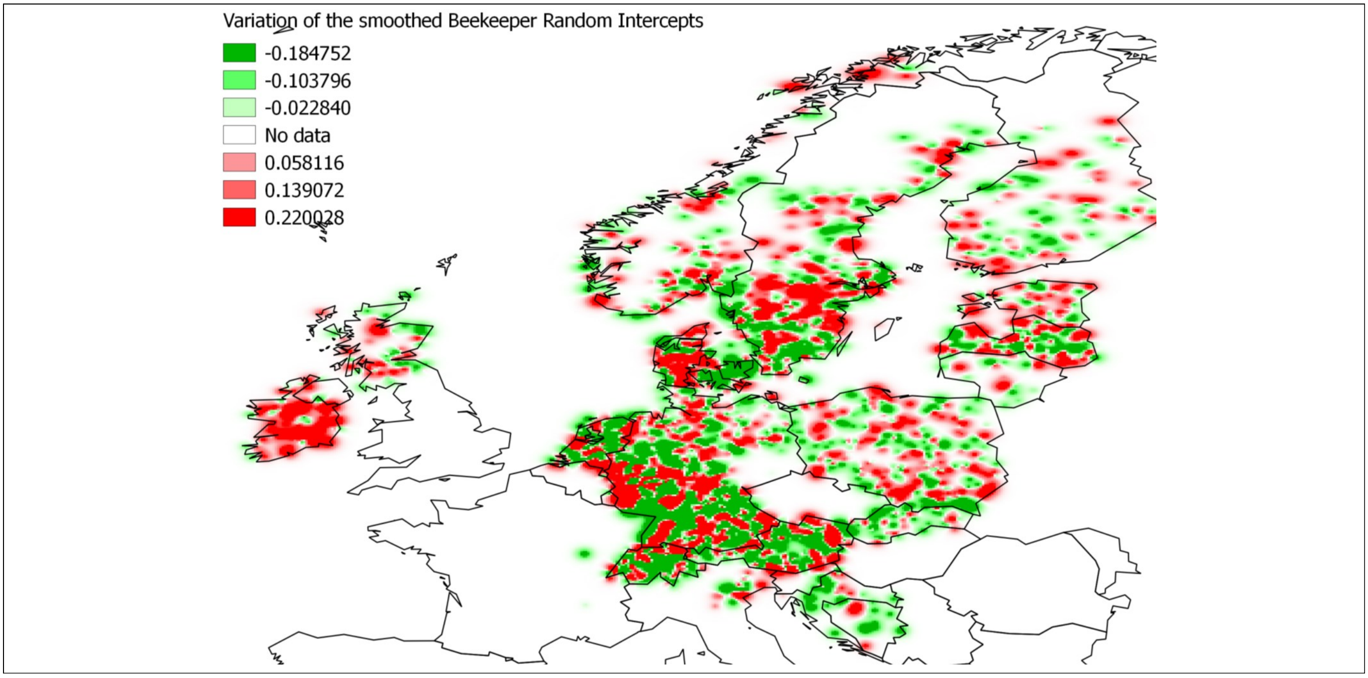

Fig. 2. Smoothed map showing the beekeeper level random intercepts in a binomial GLMM without any fixed factors for beekeeper risk of colony loss in European countries, for the 1 to 50 colony operations. White areas in the map correspond to no data, light green corresponds to values from 0 to -0.022840 inclusive, mid green represents values beyond -0.022840 to -0.103796, dark green represents values beyond -0.103796 to -0.184752, and similarly for the red coding of positive values of the random intercepts. The choice of categories used in the colour coding uses the default cumulative count option in QGIS. Darker green indicates areas of less risk and darker red areas of higher risk of winter colony loss.

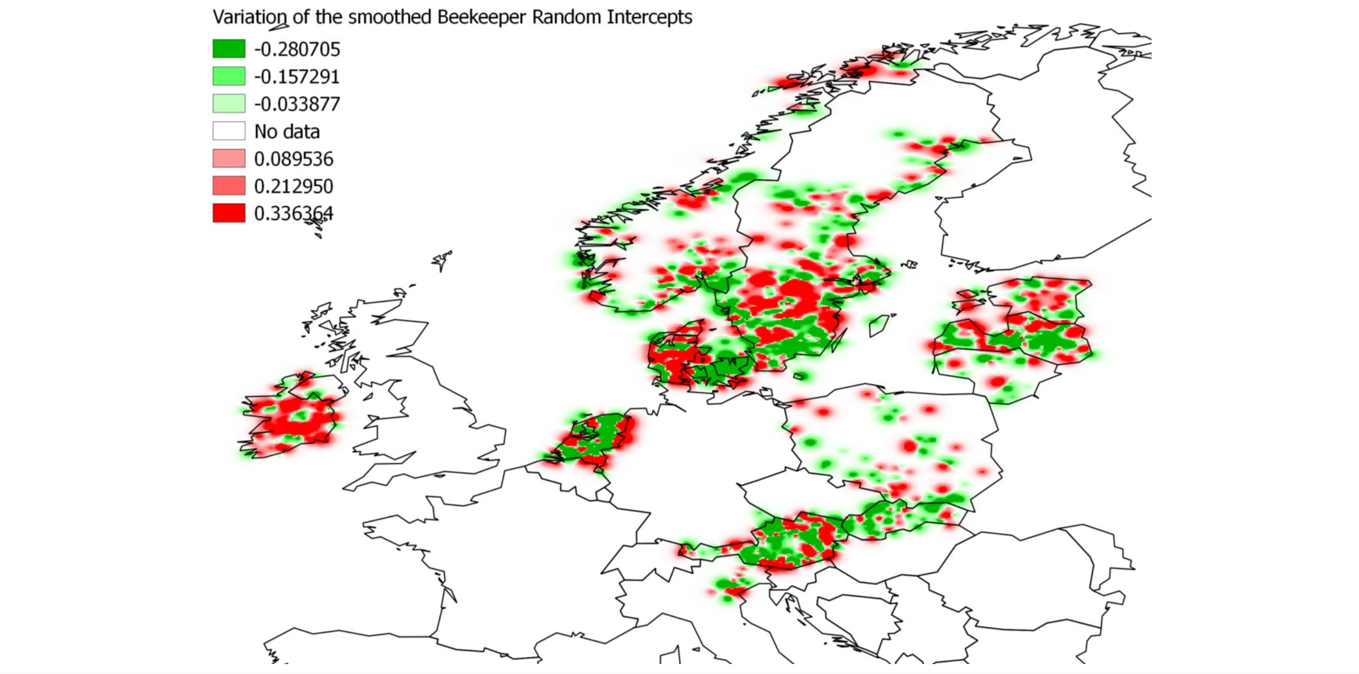

Fig. 3. Smoothed map showing the beekeeper level random intercepts in the final binomial GLMM after including the fixed factors for beekeeper risk of colony loss in European countries, for the 1 to 50 colony operations. White areas in the map correspond to no data, light green corresponds to values below 0 to -0.033877 and so on, similarly to fig. 2. The choice of categories used in the colour coding uses the default cumulative count option in QGIS. Darker green indicates areas of less risk and darker red areas of higher risk of loss.

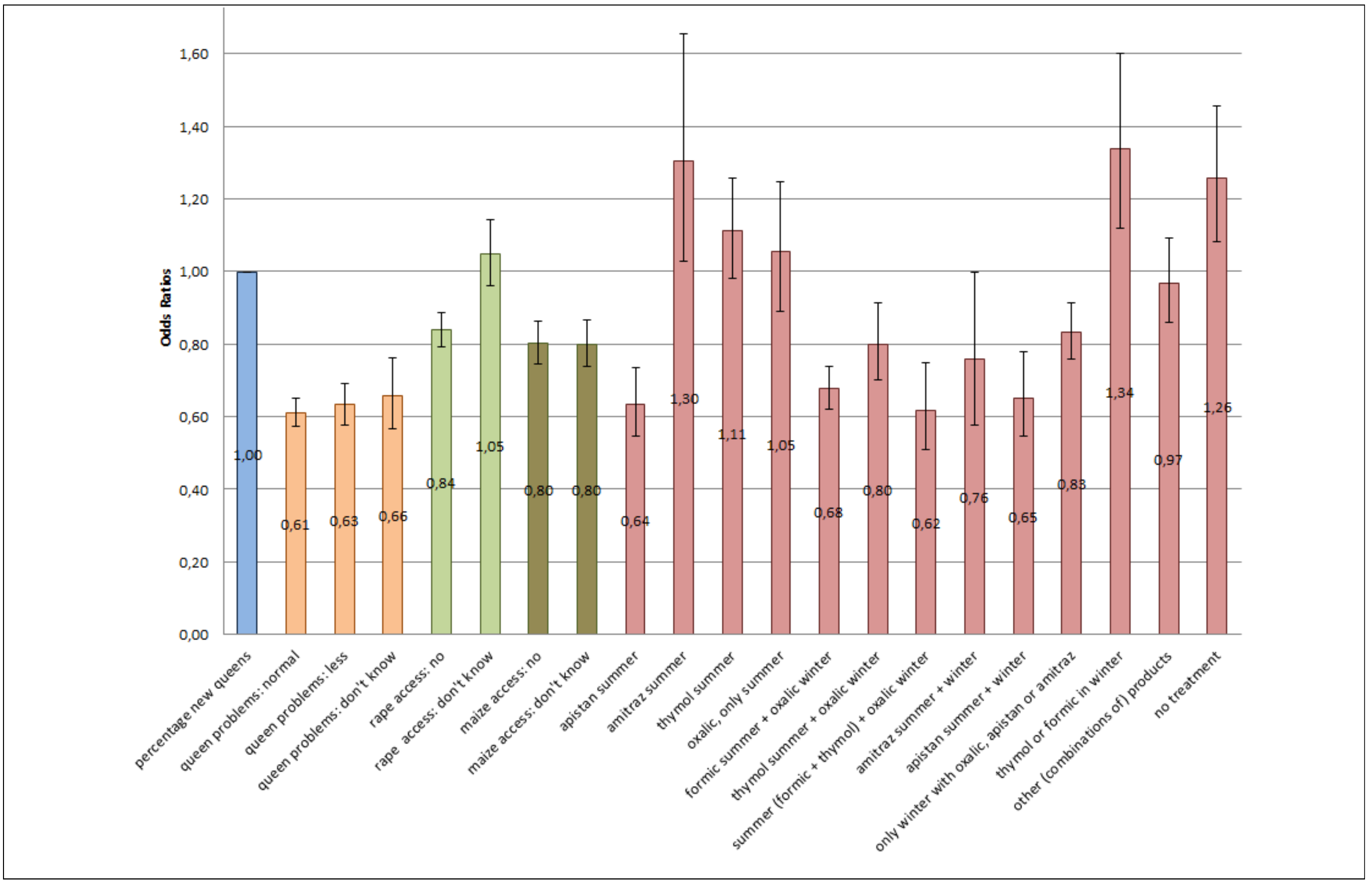

Fig. 4. Odds Ratios for fixed model factors, with 95% confidence intervals. Fixed model terms are percentage new queens, queen problems in summer 2012, access to oilseed rape, access to maize and varroa mite treatment. Baseline categories used were: more queen problems than usual (yes), access to oilseed rape (yes), access to maize (yes*)* and varroa mite only treated with formic acid in summer.

Table 7. Summary results of model fitting: AIC = Akaike Information Criterion, BIC = Bayesian Information Criterion, logLik = log Likelihood, Deviance = -2 logLik; a low AIC, low BIC, low Deviance and less negative logLik indicate a better model.

Model	**AIC**	**BIC**	**logLik**	**Deviance**
Null model with both beekeeper and region as random effects	11216	11236	-5605	11210
Final model with both beekeeper and region as random effects	11105	11264	-5528	11057
Null model with only bee-keeper as a random effect	11416	11429	-5706	11412
Full model with only bee-keeper as a random effect	11243	11396	-5599	11197
Null model with only region as a random effect	15894	15907	-7945	15890
Full model with only region as a random effect	15617	15770	-7785	15571

very similar to this and both are similar to the map shown in Fig. 2 for the countries represented. This suggests that, whilst the fixed effects do explain significant amounts of variation in the loss rates, there is still a high level of unexplained variation. Possible reasons for this and other factors to be investigated are outlined in the discussion.

Table 8. Analysis of the fixed effects in the final model: Df = Degrees of freedom for the term dropped, AIC = Akaike Information Criterion, LRT = Likelihood Ratio Test statistic for the change in model fit and the p-value of the test. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Term dropped	**Df**	**AIC**	**LRT**	**p-value**
none		11104		
percentage new queen	1	11108	5.680	0.017159 *
queenproblem2012	3	11159	60.882	3.810e-13***
oilseed rape	2	11112	12.022	0.002451 **
maize	2	11111	10.089	0.006444 **
varroa mite treatment	13	11142	63.288	1.347e-08 ***

Table 7 shows the fit of the full models with different random effects (just a beekeeper effect, just a region effect, and both of these) and the corresponding null models with an intercept only and no covariates or factors. Several measures of model fit are presented in the table. A low AIC (Akaike's Information Criterion) or BIC (Bayesian Information Criterion), a high (less negative) log-likelihood, and a low deviance (which is minus twice the log-likelihood) indicate a better-fitting model. Including only beekeeper as a random effect provides a much better fit than only including region, so there is more variation between beekeepers than between regions. However the final model with both beekeeper and region as random effects has much the best fit. In each case including the covariate and factors does explain variation and the final model gives a better fit than the null model, though beekeeper variation greatly exceeds the variation explained by both the covariate and the factors. Testing the change in model deviance shows that both beekeeper and region are highly significant effects in the model.

Table 8 shows the AIC for the final model and when each fixed effect term is dropped, one at a time, and the corresponding likelihood ratio test statistic (LRT) and its p-value. A rise in AIC and a significant p - value indicate that that term should not be dropped from the model. The most significant term in the model is queen problems, followed by varroa mite treatment, then access to rape and maize, with proportion of new queens replaced as the least significant of the variables.

Fig. 4 shows odds ratios for the risk of loss and 95% confidence intervals for these odds for the terms in the model, relative to the baseline odds. The odds ratio is very close to 1 for the proportion of new queens. However the model shows that there is a very slight reduction in the odds of loss for each percentage increase in queen replacement. Concerning queen problems, the odds of loss are lower for beekeepers with anything other than a higher than normal level of queen problems. The odds of loss are lower for colonies with no access to rape as forage compared to colonies which do, and no or unknown access to maize also reduces the risk of loss.

There are clear and statistically significant differences in the effects of varroa mite treatment strategy on the odds of loss, relative to treating only in summer with formic acid. A treatment strategy of formic acid in summer and oxalic acid in winter was performed by far the largest group of beekeepers and was one of the most effective ones for reducing the risk of winter loss. Adding a thymol product to the summer treatment seems to further reduce the risk of losses, although the difference is not significant. Another large group treated only in winter with oxalic acid, Apistan® (Tau-fluvalinate) or Amitraz and this was also more effective than the strategies of only treating in summer, with the exception of the small group who used Apistan® in summer only, which also reduced the risk of loss. Using Apistan® in summer and winter was also a successful strategy to reduce losses.

Discussion

In this study we have examined winter loss rates for many, mostly European, countries. Sadly we were not able to provide a complete European analysis in this study for all of the countries involved in the COLOSS loss monitoring. In fact in carrying out the study we experienced the nature of European co-operation between countries as can also be observed in many other fields (such as economics and politics). The extent of collaboration of the countries in implementing the standardised COLOSS questionnaire varies from no participation for some countries, partial implementation of the questions or use of the questionnaire in some regions only for other countries, to full participation of other countries, and the situation also differs from year to year. There are various reasons for this, but it affects the analysis and its outcome. Most south and east European countries, where honey production has a substantial economic impact, are absent from the countries represented in this study, or were only able to send data for a limited number of regions (for example, Italy and Bosnia-Herzegovina). Other countries collected data (England and Wales) but could not deliver it in time, or could only send a limited dataset (Croatia, and, from outside Europe, Israel). However, despite these difficulties the present study is still able to include results for many countries from North, Central and West Europe.

The nature of the surveys carried out in different countries also varies. Questionnaire data were considered as representative if the sampling was randomised (as for Scotland and some Polish regions), or was at least collected via the internet, combined with mail, email, journals or phone, with an announcement also being published in the main national beekeeper journals (as in Brodschneider *et al.*, 2010; van der Zee *et al.*, 2013). This combined approach was used by Austria, the Baltic countries (Latvia, Lithuania, Estonia), Denmark, Finland, Germany, Ireland, the Netherlands, Norway, Poland, Slovakia, Sweden and Switzerland. The question arises as to what extent the surveys of these countries which used internet surveys may be biased by coverage error. Mohorko *et al.* (2013), for example, demonstrate that coverage bias due to low internet penetration is disappearing across countries in Europe. The extent of internet coverage of the countries in the present study varied in 2013 between 64.9% (in Poland) to 90% and higher (Denmark, the Netherlands, Norway and Sweden), while the average European extent of coverage (as a percentage of the population) is 63.2% (Internet World Stats, 2013). We conclude that internet coverage may not be complete, but ranges from substantial to nearly full coverage for the countries using internet surveys for this study.

The losses of honey bee colonies during winter 2012-2013 were high (>30%) in England and Wales (British Beekeepers' Association, 2013), Scotland, Ireland, the Scandinavian and the Baltic countries. The other European mainland countries had relatively low losses compared to previous years (van der Zee *et al.*, 2012). The countries with high losses all reported extremely bad weather during the foraging season of 2012. In Ireland and Scotland, at least, this led to possible poor queen mating and weaker colonies going into winter. Another consideration is that the high regional random effect, indicating high risk of loss, seen in Fig. 1 for the north of Norway, corresponds to areas where the varroa mite has not yet been observed. It may well be the case that weather was an important contributor in explaining at least part of the losses in these cases, however this needs detailed clarification in further investigations of the available data. In the case of Norway there are few beekeepers in the remote northern parts.

Beekeepers who experienced higher queen problems than normal also suffered higher winter losses. Queen supersedure problems are not uncommon if bad weather prevents mating flights. However queen problems might also indicate existing colony health problems related to in-hive pathogens or pesticides (Pettis *et al.*, 2004; Tarpy *et al.*, 2012; Collins and Pettis, 2013), or specific queen related problems (Delaney *et al.*, 2011; Tarpy *et al.*, 2013; vanEngelsdorp, *et al.*, 2013b). We also found that young queens lowered the risk of colony loss, which is in accordance with Genersch *et al.* (2010).

Access to foraging on maize and oilseed rape were both significantly associated with winter colony loss. Maize and oilseed rape were investigated in this study because of the identification of risks to honey bees from neonicotinoid pesticides by the European Food Safety Authority (EFSA) (2013). The EFSA position was based on a risk assessment of the exposure of honey bees to contaminated maize through guttation fluid. There are many studies establishing sub-lethal effects, for example Hatjina *et al.* (2013) and references cited there. Cresswell (2011) estimated from a meta-analysis of 14 published studies that field-realistic levels of imidacloprid would have sub-lethal effects on honey bees that reduced their expected performance by up to 20%. However the publications on the impact of pesticides on colony health are still very contradictory. Nguyen *et al.* (2009) found that winter colony loss was inversely associated with foraging in maize fields treated with imidacloprid. Genersch *et al.* (2010) did not find any association between foraging on oilseed rape and winter losses. Henry *et al.* (2012) demonstrated in an experimental study that exposure of foragers to non-lethal concentrations (as used on oilseed rape) of thiamethoxam can affect forager survival, with potential contributions to the risk of colony collapse. The authors claimed that the concentrations used were field-realistic, but this is arguable and these concentrations may well have been higher than would be encountered in the field. Cresswell and Thompson (2012) comment on the conclusions of Henry *et al.* (2012), raising arguments from study results which suggest that "dietary thiamethoxam would not precipitate collapse in healthy colonies in spring, but this does not rule out the possibility that colonies will be more vulnerable later in the year when their capacity to replace lost workers has diminished."

An observational study such as the present one can indicate associations between reported access to some types of forage and honey bee colony health, but it is important to emphasise that the associations found cannot themselves indicate a causative link. In fact a causative explanation can only be found in carefully designed risk assessment studies at colony level. Also, other factors such as nutrient-quality of pollen available to honey bee colonies could play a role in our findings (Brodschneider and Crailsheim, 2010; Höcherl *et al.*, 2012). Rather we can derive epidemiological statements on the quality of habitats available for honey bee colonies. The presence of maize and rape could well be an indication of an extensively used monoculture, as opposed to a diversity of forage, which may in itself not be beneficial for honey bees. We also emphasise that a questionnaire measures the perception of the responding beekeeper, which may be influenced by cognitive dissociation, i.e. if they take a particular position in the current debate about the effect of pesticides on honey bees.

For constructing the maps of random effects at beekeeper level, in general we used beekeeper location but we used apiary location in cases where beekeeper location was not available. In the case of a beekeeper with more than one apiary, this approach is unsatisfactory unless these apiaries are very near to each other. Also, the standardised questionnaire collects apiary level information and does not ask about migration of colonies, but migration is relevant for access to maize or oilseed rape. The model fitting included beekeepers with up to 50 colonies. In some countries, a substantial number of these beekeepers will participate in paid pollination contracts (and even more of the larger scale beekeepers not included in the model will do so). Some colonies may be migrated and others not, or colonies could be migrated to different locations. Equally, taking account of splitting and merging of colonies in summer would be relevant for migration. These issues are difficult to deal with in an observational study such as this one, but may have an effect on the results.

This study demonstrates that beekeepers who treated the varroa mite in summer and winter experienced lower risks of winter loss. A varroa treatment strategy with formic acid in summer and oxalic acid in winter was by far the most commonly used product combination in the strategies used by the beekeepers in our dataset. Adding a treatment with a thymol product, as done by a limited group of beekeepers, slightly increased the effectiveness of this strategy although the difference is not significant. Another large group of beekeepers treated varroa only in winter, when brood is absent or minimal and all mites are phoretic and vulnerable to a treatment product. Results for this group show that this approach is significantly less effective than the above mentioned strategies, but still significantly better than the strategy of 'only treatment in summer with formic acid' which we used as a baseline for comparison of treatments.

Some beekeepers treated but provided no information on the treatment which they used. Others provided information that was not clearly interpretable, for a variety of reasons. Not providing details of the treatment may be due to the use of products which are unlicensed in some countries (Mutinelli and Rademacher, 2003; Mutinelli, 2006) and may be an example of nonresponse error due to the sensitive character of the question. In some countries the mite has not yet been found everywhere, so for some beekeepers varroa treatment is not applicable. These are issues to be addressed for future survey questionnaires.

Beekeeper effects depend to some extent on regional factors. Including a region level random effect in the model allows to some extent for this. However there are important regional fixed effects which should be considered in further model development, especially concerning meteorological and land-use data. The effect of varroa treatment is important, but treatments are sometimes associated with a particular country or region, e.g. the general use of thymol in summer as the only treatment in Ireland which was reported in the data used for this study, so that the effects of treatment and country interact. The outcome of treatment will depend to some extent on environmental conditions such as weather in that region or country, which will vary from year to year. Agricultural data may give further indications of areas where biodiversity may be low or agricultural pesticides may play a role. Meteorological data can further explain if opportunities to use sufficient available forage were limited, which seems to have been the case in the North European countries. A further development of model fitting will also be to include random slopes in the models, which is another way to allow for differences between regions in the effect of the co-variates and factors used in the models.

A related issue is that we are modelling the risk of any winter loss. In the standardised questionnaire for 2013 the beekeeper decided when colonies were prepared for winter and when winter finished, for the purposes of stating colonies kept at the start of the winter and colonies lost over winter. Length of winter of course varies from country to country and also within countries, however so also do weather conditions and temperature for example. Northern countries also have a shorter summer, resulting in a shorter brood period and fewer mite reproduction cycles, leading to less varroa-related risk of loss. These are examples of regional factors that could be included in a model as explanatory variables. This will also be pursued in further work.

Concerning the model fitting, generalised linear modelling provides a powerful framework for the investigation of the effect of multiple possible risk factors on colony losses. Mixed models incorporating random effects, as well as the covariates and factors of interest, allow for the hierarchical structure of data collected within countries and regions as well as for differing effects at beekeeper level. In our mixed model analysis we investigated to what extent the significant model factors explain losses. The variation in loss rates between beekeepers and regions decreases and the model fit improves after adding these factors to a null model, but this gives only a limited explanation since the unexplained variation (random effects) remain at a high level. Mapping the random intercepts in these fitted models gives a spatial view of this unexplained variation after allowing for significant risk factors. This in turn focusses attention on areas where the unexplained losses are high and where further investigation may be useful. The variation between beekeepers (Fig. 3) gives an indication of where such areas may exist.

Large scale observational studies such as the present study can contribute in at least two important respects to the clarification of colony losses. Identification of geographical areas of higher risk allows for a spatially stratified sampling design in studies which investigate determinants for winter loss at colony level. Additionally, whereas experimental studies in general neutralise the role of the beekeeper, observational studies such as this one recognise and estimate the important variation in colony loss between beekeepers.

To summarise, we have presented colony loss rates for 19 countries, although these are not in all cases country-wide. Using generalised linear models we have been able to identify several risk factors. Bee-keeping management is important, as we have shown for the manner of treatment against the parasitic mite *Varroa destructor* and concerning the recognition and correction of queen problems. We have also identified environmental factors using this epidemiological approach, namely that access to certain types of agricultural crops available to honey bees has been demonstrated to increase the risk of colony mortality. The detailed causes for this remain unclear and might be found by further investigation of habitat type, nutritional value of crops or treatment with pesticides. Finally, we have identified risk areas at regional level and were able to visualise the model random intercepts, representing unexplained risk at beekeeper level, to obtain an impression of where future spatial analysis may reveal clustering at a higher resolution not restricted by administrative boundaries. Further work will include confirming the importance of the risk factors found for losses in winter 2012-2013 by examining several years of loss data, and will investigate additional variables which may be relevant for explaining some of the regional variation in losses not accounted for so far.

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