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Genomics reveal local skin immune response key to control sarcoptic mange in Iberian ibex (*Capra pyrenaica*)

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Abstract

Background Sarcoptic mange is an emerging and neglected contagious skin disease caused by the mite *Sarcoptes scabiei*, affecting humans, domestic animals, and wildlife. Mange is the main disease and a major concern for the management and conservation of populations of Iberian ibex (*Capra pyrenaica*), a medium-sized mountain ungulate endemic to the Iberian Peninsula and Northern Pyrenees. Differences in host-parasite interaction and host immune response determine mange clinical outcome, but little is known about the related differences in gene expression. This study determined blood and skin gene expressions in *S. scabiei*-experimentally infested Iberian ibexes.

Results Infestation with *S. scabiei* promoted immune and inflammatory genomic responses both in skin and blood, with two different clinical outcomes: either severe infestation or recovery. *Sarcoptes scabiei* induced local skin immunosuppression to favour its multiplication and establishment of the infestation in the host. Skin gene expression was mostly inflammatory and inefficient to control mange in the severely infected ibexes. Conversely, the immune skin response of the recovered ibexes effectively recognised *S. scabiei* and activated T-cells, limiting the infestation. Consequently, inflammation-related genes were more expressed in the blood of the severely infested ibexes than in those that recovered.

Conclusions The results demonstrate that skin local cellular immune response is key to control sarcoptic mange and prevent the systemic spread of the disease and the associated inflammatory response. These results will be useful to understand the pathogenesis and drivers of the differential outcome of mange at individual scale, and the population and ecological consequences of such variability in Iberian ibex, as well as in other wildlife species, domestic animals, and humans.

Keywords Gene expression, Gene set enrichment analysis, Genomic response, Immune response, Microarray, *Sarcoptes scabiei*

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Background

Sarcoptic mange is a contagious skin disease caused by the mite *Sarcoptes scabiei*. It is considered an emerging, reemerging and neglected disease in humans, domestic animals, and wildlife [1–6]. *Sarcoptes scabiei* is a generalist parasite capable of establishing and sustaining transmission in different host species and populations through host immune response modulation, on-host movement capacity, off-host seeking behaviour, and environmental persistence [7]. Mange can course with pruritus, scaling, alopecia, erythematous crusted papules, hyperkeratosis, crust formation and, in chronic lesions, lichenification, known in humans as Norwegian or crusted scabies. However, a mild slightly symptomatic form known as ordinary scabies is more frequent in humans [8–10]. Such differences in the clinical presentation of the disease are not related to variability in mite pathogenicity but to differences in the capability of the immune response of the host to cope with the infestation [8, 11, 12]. However, the gene expression bases of differential immune response in scabietic hosts have only been limitedly investigated in domestic animals and humans [13–17], although gene expression studies can contribute to advance in the knowledge of the pathogenesis of diseases [18–21].

In wildlife, the individual clinical expression and mortality outcome and, consequently, the demographic effects of diseases in free-ranging populations can be related to interindividual differences of the host, the pathogen and/or the environment [22]. Sarcoptic mange has been reported in different wild ungulate species in Europe, including chamois (*Rupicapra* spp.) [6, 23–25], both Alpine and Iberian ibex (*Capra ibex* and *C. pyrenaica*, respectively) [6, 24, 26–29], Barbary sheep (*Ammotragus lervia*) [24, 30], mouflon (*Ovis gmelini*) [24, 31], fallow, red, and roe deer (*Dama dama*, *Cervus elaphus*, and *Capreolus capreolus*, respectively) [24, 31–33], and wild boar (*Sus scrofa*) [34, 35]. However, the host-mite interaction established at individual scale will determine differences among host species, and even among populations of the same species, in the epidemiological, mortality, demographic, and genetic consequences of sarcoptic mange in wildlife [7, 36, 37].

Among the aforementioned species, Iberian ibex is a valuable mountain ungulate as an Iberian Peninsula endemism and a big game species [38, 39]. Sarcoptic mange is considered the main health concern for its management and conservation [29, 40]. High mortality rates related to sarcoptic mange epizootics have been reported in Iberian ibex populations, with varying demographic consequences in the populations affected [26, 41–44]. Moreover, resistance and survival to sarcoptic mange have been reported in free-ranging ibexes naturally infested from Sierra Nevada Natural and National Park (SNNNP) [45, 46], and in experimentally-infested ibexes as well [11, 47, 48]. However, the individual intrinsic factors for such different outcomes in resistance and the demographic population consequences in Iberian ibex are mostly unknown, including gene expression. Gene expression studies are required to provide insight on the pathogenesis of sarcoptic mange in Iberian ibex, to advance in the knowledge of the pathogenesis and population consequences of this disease in this species but also as a model for other wildlife species, domestic animals, and even human genomic response to *S. scabiei* infestation.

The aims of the study are: (1) to characterize skin and blood gene expressions in Iberian ibexes experimentally infested with *S. scabiei*; and (2) to identify the biological processes and pathways determining the differences in the clinical outcome to sarcoptic mange in Iberian ibex.

Materials and methods

Experimental infestation with *S. scabiei*

Eighteen Iberian ibexes (ten females one- to six-year-old and eight males one- to three-year-old, Table 1) from a stock reservoir protected from exposure to *S. scabiei* located in SNNNP [48, 49] were moved to "Las Mimbres" facilities at Sierra de Huétor Natural Park (Granada, Spain). After an eight-week adaption period, 12 of the Iberian ibexes were infested with *S. scabiei* using skin pieces of a naturally parasitized wild ibex, whereas the remaining six ibexes were maintained as controls. The scabietic skin pieces were attached with elastic bandages to the inter-scapular region, previously shaved to induce contact between the mites and the host skin. The mite load in the skin pieces applied to each ibex was 750 ± 440 mites, estimated according to previously reported protocols [50, 51].

Clinical assessment and sample collection

Clinical signs, including the presence of mangy skin lesions and the skin surface affected, were monitored during the 150-day infestation study period. The percentage of the whole-body skin surface affected by lesions compatible with sarcoptic mange was visually estimated as previously reported [50, 52–54]. By day 131 post-infestation, whole blood samples were collected from the jugular vein in commercially available tubes with ribonucleic acid (RNA) preservation buffer (PAXgene™ blood RNA tubes, QIAGEN, Hilde, Germany). Skin biopsies were also obtained the same day using an 8-mm diameter punch biopsy tool and were placed in disposable tubes with RNA*later*® (Thermo Fisher Scientific, Waltham, MA, USA) solution. The samples were stored at -80ºC until analysis. According to the protocol approved by the corresponding animal ethics committee and following

Table 1 Iberian ibexes (*Capra pyrenaica*) experimentally infested with *Sarcoptes scabiei* and sampled for genomic analysis

Experimental group		Identification	Sex	Age (years)	Percentage of skin lesions at sampling (day 131 post-infestation)
Control		10	Female	3	0
		12	Female	2	0
		23	Male	1	0
		27	Female	6	0
		35	Male	3	0
		45	Female	4	0
Infested	**Recovered (≤ 25% skin lesion)**	9	Male	5	25
		29	Female	4	5
		40	Male	1	3
		44	Male	2	0
	Mean				8.25
	Severely infested (> 70% skin lesion)	4	Female	6	100
		7	Female	2	70
		8	Male	4	90
		18	Male	3	700
		20	Female	2	80
		31	Female	1	100
		33	Male	3	70
		43	Female	3	100
	Mean				88.75

European and Spanish legislation (see Ethics approval section), at day 150 post-infestation 8 of the 12 experimentally infected ibexes were euthanized due to their severe terminal mange condition (Table 1). The ibexes were anaesthetized by intramuscular injection with a mixture of xylazine (3 mg/kg) and ketamine (3 mg/kg) [11, 55]. Once unconscious, they were euthanized with an intravenous injection of T-61® (embutramide 12 mg/kg, membezonium iodide 3 mg/kg, tetracaine 0.3 mg/kg) [11].

Gene expression analysis

RNA was extracted using the PAXgene kit (QIAGEN, Hilde, Germany) for blood samples and the RNeasy mini kit (QIAGEN, Hilde, Germany) for skin samples. The samples were processed according to the following Affymetrix protocols: GeneChip WT PLUS Reagent kit (P/N 703174 Rev. 2) and Expression Wash, Stain and Scan User Manual (P/N 702731 Rev. 3) (Affymetrix Inc., Santa Clara, CA, USA). Gene expression was measured with microarrays using the Ovine Gene 1.0 ST array (Affymetrix™, Santa Clara, CA, USA), and genes were noted using the annotation file provided by Affymetrix for the OviGene-1.0 ST array.

After processing, the data were quality-controlled and normalized using the Robust Multi-array Average algorithm (RMA) [56] included in the *aroma.affymetrix* package [57]. The statistical analyses were performed using R software version 3.5.0 [58] and Bioconductor packages [59]. Linear models were used to assess differential gene expression among the different clinical outcomes with the *limma* package [60]. The *sva* Package [61] from Bioconductor was used in order to estimate batch and other artefacts into surrogate variables which were included as covariates into the limma model.

Differences in gene expression were studied between the control and the infested ibexes (as a whole and for each clinical outcome separately), and between the recovered infested ibexes and those severely infested. Genes were considered differentially expressed when the *p*-value was lower than 0.05 and the *log* fold-change was higher than 0.58 or lower than -0.58, corresponding to an absolute value of fold change over 1.5 [62, 63].

Gene Set Enrichment Analysis (GSEA) was used in order to retrieve functional pathways. This method links the obtained gene expression profile with gene sets available in the Molecular Signatures Database (MSigDB) [64, 65]. Gene sets in MSigDB are grouped in different collections, from which C2 KEGG (Kyoto Encyclopaedia of Genes and Genomes) curated gene sets and C5 GO (Gene Ontology) biological process were used in this study. The GSEA software was used with the option

Pre-Ranked Analysis to rank genes using the *p*-values obtained in the Differential Expression analysis with *limma* package. The ranked list of genes was generated using the score [-*log*(*p*-value)*sign(fold change)] for each gene. The gene sets were filtered by a nominal *p*-value of 0.05 and a false discovery rate (FDR, *q*-value) of 0.1.

The differences in the functional pathways were investigated among the same groups as the differences in gene expression using the GO biological process collection. Moreover, the KEGG collection was used to further investigate the differences between the recovered infested ibexes and those severely infested.

Results

Clinical signs

None of the control ibexes developed skin lesions. At sampling on day 131 post-infestation, four of the infested ibexes developed skin lesions in less or equal than 25% of the body surface, which then decreased to even achieve complete healing (recovered ibexes). Conversely, at sampling the remaining eight ibexes had developed lesions in more than 70% of the skin surface, which continue to progress in all cases (severely infested ibexes) (Tables 1 and 2, Supplementary material Fig. 1).

Gene expression

Table 3 and Supplementary material Fig. 2 show the numbers of up-regulated and down-regulated genes found for each of the comparisons performed (infested *versus* control ibexes; recovered *versus* control ibexes, severely infested *versus* control ibexes; and, finally, recovered *versus* severely infested ibexes). A significant percentage of these genes were related to the immune response to the mite *S. scabiei* and the related lesional and inflammatory skin disorder (Table 3, Supplementary material Fig. 2). Thus, the genes up-regulated in the infested ibexes altogether as compared to the controls included interleukins 36, beta and gamma; chemokine (C–C motif) ligands 7, 8 and 20 and receptors 1 and 2; interleukin receptors 13 and 15; interleukin-8-like; and lactotransferrin, among others (Supplementary material Table 1). However, the number of differentially expressed genes as compared to controls was higher for the severely infested than for the recovered ibexes (Table 3, Supplementary material Fig. 2). When comparing the recovered and the severely infested ibexes, two up-regulated genes related to antigen presentation could be identified both in skin and blood of the recovered ibexes, namely SLA class II histocompatibility antigen and DQ haplotype D alpha chain-like (Supplementary material Table 2). The 260 up-regulated genes

Table 2 Percentage of the whole-body skin surface affected by skin lesions compatible with sarcoptic mange in the control, recovered and severely infested Iberian ibex (*Capra pyrenaica*) experimentally infested with *Sarcoptes scabiei*

	Days post-infestations											
	0	**4**	**13**	**26**	**33**	**46**	**61**	**75**	**103**	**120**	**131**	**150**
Control												
C1	0	0	0	0	0	0	0	0	0	0	0	0
C2	0	0	0	0	0	0	0	0	0	0	0	0
C3	0	0	0	0	0	0	0	0	0	0	0	0
C4	0	0	0	0	0	0	0	0	0	0	0	0
C5	0	0	0	0	0	0	0	0	0	0	0	0
C6	0	0	0	0	0	0	0	0	0	0	0	0
Recovered												
R1	0	0	1	1	1	5	10	15	20	25	25	15
R2	0	0	0	1	3	5	15	15	10	10	5	3
R3	0	0	1	3	3	5	10	10	5	5	3	0
R4	0	0	1	3	5	5	0	0	0	0	0	0
Severely infested												
SI1	0	0	0	3	3	5	25	55	100	100	100	100
SI2	0	0	0	1	1	5	10	15	30	70	70	85
SI3	0	0	0	1	3	5	15	20	80	90	90	95
SI4	0	0	0	3	3	5	15	20	85	95	100	100
SI5	0	0	0	3	3	5	10	20	30	30	80	90
SI6	0	0	1	5	10	15	20	50	80	100	100	100
SI7	0	0	0	5	5	10	10	15	30	40	70	85
SI8	0	0	1	1	3	5	25	50	100	100	100	100

Table 3 Genes differentially expressed among the groups of Iberian ibexes (*Capra pyrenaica*) with different sarcoptic mange evolution

Expression change as compared to controls												
	SKIN						**BLOOD**					
	Up			**Down**			**Up**			**Down**		
	TOTAL	Immune	Lesion and inflammation	TOTAL	Immune	Lesion and inflammation	TOTAL	Immune	Lesion and inflammation	TOTAL	Immune	Lesion and inflammation
Infested altogether	141	18 (12.8%)	30 (21.3%)	295	15 (5.1%)	30 (10.2%)	124	24 (19.4%)	3 (2.4%)	92	22 (23.9%)	3 (3.3%)
Recovered	88	7 (8.0%)	23 (26.1%)	79	4 (5.1%)	7 (8.9%)	52	9 (17.3%)	2 (3.8%)	73	17 (23.3%)	3 (4.1%)
Severely infested	233	30 (12.9%)	28 (12.0%)	493	18 (3.7%)	69 (14.0%)	309	41 (13.3%)	26 (8.4%)	150	31 (20.7%)	8 (5.3%)
Expression change as compared to severely infested												
	SKIN						**BLOOD**					
	Up			**Down**			**Up**			**Down**		
	TOTAL	Immune	Lesion and inflammation	TOTAL	Immune	Lesion and inflammation	TOTAL	Immune	Lesion and inflammation	TOTAL	Immune	Lesion and inflammation
Recovered	260	23 (8.8%)	57 (21.9%)	143	11 (7.7%)	11 (7.7%)	53	16 (30.2%)	0 (0.0%)	242	28 (11.6%)	25 (10.3%)

in the skin of the recovered ibexes also included genes related both to cellular immunity, such as chemokine (C–C motif) ligand 22, lymphoid enhancer-binding factor 1, HLA class II histocompatibility antigen, and DQ alpha 2 chain-like, and to inflammation and allergic response, such as interleukin-37-like; interleukin 1, Alpha; interleukin 33; major allergen I polypeptide chain 1-like; major allergen I polypeptide chain 1-like; interleukin 2 receptor, beta; chemokine (C-X-C motif) ligand 9, 10 and 11, or cysteinyl leukotriene receptor 1. In blood, the 53 genes up-regulated in the recovered as compared to the severely infested ibexes included 16 immunity-related ones, such as T-cell receptor-associated transmembrane adapter 1, T-lymphocyte surface antigen Ly-9-like, natural killer cells antigen CD94-like, putative killer cell immunoglobulin-like receptor like protein KIR3DP1 or SH2 domain containing 2A (Table 3, Supplementary material Fig. 2, Supplementary material Table 2). Conversely, both in skin ($n=143$) and blood ($n=242$) the genes down-regulated in the recovered ibexes as compared to the severely infested ones were mostly related to inflammation, such as interleukines (e.g., interleukins 18 and 36) or acute-phase proteins (e.g., serum amyloid A protein; Table 3, Supplementary material Fig. 2, Supplementary material Table 2).

Tables 4 and 5, and Supplementary material Figs. 3 and 4 show the differences in the functional pathways assessed using the GO biological process collection between the infested ibexes (altogether and for the recovered and severely infested separately) and the control ones, as well as between the recovered and the severely infested ibexes. Additionally, Tables 6 and 7, Supplementary material Figs. 5 and 6 show the differences between the recovered infested ibexes and those severely infested as investigated using the KEGG collection.

Discussion

To the authors' knowledge, this is the first study on genomic response to sarcoptic mange in a wild host species. Up to date, the modulation of gene expression by *S. scabiei* has been previously studied only in mice spleen [13], human skin equivalents [14], rabbit skin [15], a porcine model [16], and human skin [17]. *Sarcoptes scabiei* induced both up-regulation and down-regulation of gene expression related with immune response, inflammation, and tissue disorder both in the skin and blood of the infested Iberian ibexes, overall agreeing with previous descriptions in rabbit and human skin [15, 17]. Moreover, differences in the local skin immune response gene expression among the infested ibexes were related to differences in the systemic blood gene expression and determined the clinical outcome, agreeing with previous clinical and histopathological reports [11, 48]. As previously reported in a porcine model of crusted versus ordinary scabies [16], *S. scabiei* infestation promoted immune and inflammatory response in this species, both in skin and in blood, and was associated with a lower expression of genes related to cell division, such as fibroblast growth factors 5 and 23 (Supplementary material Table 1). The ibexes that recovered from mange had milder skin local and blood systemic inflammatory and immune responses, and higher cell activity in skin than the severely affected ones (Supplementary material Table 3). In addition, genes related to immunity were more expressed in the skin of the recovered ibexes than in the skin of the severely infested and the control groups. Conversely, in blood immunity-related genes were more expressed in the severely infested ibexes than in the recovered and control ones (Supplementary material Tables 2, 3, and 4).

This study further confirms that Iberian ibexes can spontaneously control *S. scabiei* infestation and even recover under experimental infestation conditions, agreeing with the results already reported both for free-ranging Iberian ibexes in the SNNNP [29, 45, 46] and experimentally-infested ibexes [11, 47, 48]. However, the pathogenesis of sarcoptic mange and the immune mechanisms leading to resistance against *S. scabiei* in Iberian ibexes are not yet fully understood.

As compared with the recovered and the control ibexes, the overall pro-inflammatory and immune response profile found in the severely infested Iberian ibexes was consistent with an ineffective immune response against the mite and with the activation of defence response against secondary bacterial infections [11, 66–68]. Beyond defence against bacteria, the processes up-regulated in the severely infested ibexes included innate immune response genes, such as CD14, 2–5-oligoadenylate synthetase 1, radical S-adenosyl methionine complement component 7 lysozyme C-3-like. In humans, the clinical manifestations of severe scabies have been associated with a marked Th2 allergic immune response against the mite and its secretory products, such as salivary enzymes, eggs and faeces [69, 70]. The up-regulated gene sets associated with inflammation, cytokine production and response, mast cells and interferons found in the severely affected ibexes, agree with this link between the allergic and inflammatory responses and the lesions [8, 71]. These gene sets included interleukins 18, 19, 36, several interleukin receptors, chemokine ligand 8 and receptors 1 and 2, interferon induced transmembrane protein 3 and very large GTPase 1-like, lactotransferrin, CD14, CD163 and CD33 molecules, and tumour necrosis factor alpha-induced protein 6, among others (Supplementary material Tables 2 and 3). These results are consistent with the pro-inflammatory gene expression found in keratinocytes

Table 4 Number of differentially enriched gene sets and percentage included in pathogenically significant processes

	Expression changes as compared to controls				
		SKIN		BLOOD	
		n	Biological categories	n	Biological categories
Infested altogether	Up	26	Immunity and inflammation (65%): - Response to cytokine (23%) - Response to other organisms (15%) - Immune system processes (15%) - Cytokine production (8%) - Inflammatory response (4%)	383	Immunity and inflammation (22%): - Response to cytokine (1%) - Response to other organisms (2%) - Immune system processes (9%) - Cytokine production (5%) - Inflammatory response (2%) Response to wounding (3%)
	Down	209	Cell division (32%): - Chromosome organization (10%) - DNA metabolic processes (12%) - RNA metabolic processes (1%) - Cell cycle (8%) - Cell division (1%) Pigmentation (4%) Response to radiation (1%) Immunoglobulin production (2%)	316	Cell division (50%): - Chromosome organization (8%) - DNA metabolic processes (14%) - RNA metabolic processes (20%) - Cell cycle (3%) - Gene expression (5%) Response to radiation (3%)
- Recovered	Up	21	Immunity and inflammation (24%): - Leukocyte differentiation (19%) - Response to chemokine (5%) Cell division—Gene expression (14%) Protein folding (19%) Response to topologically incorrect protein (19%)	87	Immunity and inflammation (2%): - Leukocyte migration (1%) - Response to other organisms (1%) Response to wounding (2%) Coagulation (5%)
	Down	50	Cell division (16%): - Chromosome organization (8%) - DNA metabolic processes (8%) Metabolic processes (62%) Pigmentation (12%)	306	Cell division (41%): - Chromosome organization (7%) - DNA metabolic processes (7%) - RNA metabolic processes (21%) - Cell cycle (1%) - Gene expression (5%) Response to radiation (1%) Immunity and inflammation (11%): - Immune system processes (9%) - Cytokine production (2%)
- Severely infested	Up	14	Immunity and inflammation (79%): - Response to cytokine (29%) - Cytokine production (14%) - Response to other organisms (29%) - Lymphocyte migration (7%)	249	Immunity and inflammation (22%): - Cytokine production (8%) - Leukocyte processes (7%) - Innate immune response (3%) - Inflammatory response (2%) - Response to other organisms (2%) Response to wounding (2%) Coagulation (1%)
	Down	209	Cell division (36%): - Cell cycle (11%) - Cell division (1%) - Chromosome organization and segregation (11%) - DNA metabolic processes (10%) - RNA metabolic processes (3%) Immune system processes (2%) Pigmentation (4%)	700	Cell division (62%): - Chromosome organization (9%) - DNA metabolic processes (20%) - RNA metabolic processes (24%) - Cell cycle (4%) - Gene expression (5%) Metabolic processes (13%) Immune system processes (4%) Response to radiation and UV (3%) Pigmentation (1%)

The differences are presented between the control and the infested Iberian ibexes (*Capra pyrenaica*), both as a whole and for each of the different clinical outcomes. Data obtained using the GO biological process collection in the GSEA analysis (nominal p-value < 0.05 and FDR < 0.1) both in skin and blood

and fibroblasts observed both in the infested ibexes of this study and in human skin equivalents in response to *S. scabiei* [14].

Serum immunoglobulin concentrations have been reported to increase with mange severity in Iberian ibexes, but they seem to indicate exposition to the mite rather than having a protective effect [66, 72]. Such ineffective antibody response is paralleled by an increase in acute phase protein concentrations, an additional indicator of the inefficient inflammatory response [73, 74].

Table 5 Number of differentially enriched gene sets and percentage included in pathogenically significant processes

	Expression changes as compared to severely infested				
	SKIN			BLOOD	
		n	Biological categories	n	Biological categories
Recovered	Up	79	Cell division (48%): - Chromosome organization (11%) - DNA metabolic processes (15%) - RNA metabolic processes (13%) - Cell cycle (6%) - Cell division (3%) Immunity—B cell proliferation (1%) Pigmentation (3%)	0	
	Down	48	Immunity and inflammation (27%): - Cytokine production (13%) - Response to cytokine (2%) - Response to other organisms (6%) - Immune system processes (4%) - Inflammatory response (2%) Metabolic processes (56%): - Lipid (21%) - Carbohydrate (10%) Lipid storage (4%)	330	Immunity and inflammation (36%): - Cytokine production (12%) - Response to cytokine (2%) - Response to other organisms (2%) - Immune system processes (11%) - Innate immune response (5%) - Adaptive immune response (1%) - Inflammatory response (2%) Response to wounding (1%)

The differences are presented between the recovered Iberian ibexes (*Capra pyrenaica*) and those severely infested. Data obtained using the GO biological process collection in the GSEA analysis (nominal p-value < 0.05 and FDR < 0.1) both in skin and blood

Accordingly, genes and pathways related to inflammation were up-regulated in the severely infested ibexes as compared to those that recovered, including acute-phase proteins such as serum amyloid A protein in skin and haptoglobin in blood (Supplementary material Table 2). The increased expression of genes related to immunity found in the skin of the recovered ibexes as compared to the enriched pathways of the severely infested ibexes suggests that local cellular immunity plays a significant role in controlling the extension of mange and the associated systemic inflammation. These immune-related genes include natural killer cells antigen CD94-like, mast cell protease 3-like, chemokine ligands 5, 9, 10, 11, and 22, interleukins 1, 33, and 37, among others (Supplementary material Table 2). This agrees with previous studies suggesting that the effective immune response to sarcoptic mange is cell-mediated [8, 11, 48, 75]. Particularly, the most enriched pathways in the skin of the ibexes that recovered, "Antigen processing and presentation" and "T-cell receptor signalling pathway" (Tables 5 and 6, Supplementary material Figs. 4 and 5), further suggest that the appropriate recognition of *S. scabiei* by the host cellular immune system is essential to control the spread of the infestation. Sarcoptic mange-related changes in immune cellular populations in skin may vary among species and clinical pictures, and include effective immune response mediated by CD4+T cells in humans [8] or a significantly increased number of B lymphocytes in the skin of the most affected Alpine chamois (*Rupicapra rupicapra)* [76]. Conversely, in other species like foxes (*Vulpes vulpes*) [77], free-living wombats (*Vombatus ursinus*) [78] or even in humans with crusted scabies [71], no plasma cells or B lymphocytes were found. These findings agree with the lower number of plasma cell previously reported in the skin of the infested ibexes of this study using histopathological and immunohistochemical techniques, as compared to the skin of non-infested ibexes [11, 48]. An exacerbated but effective local skin Th1-type cellular immune response involving T-lymphocytes seems key to control sarcoptic mange in Iberian ibex [48], agreeing with the higher expression of T-cell related genes, such as T-cell receptor-associated transmembrane adapter 1 and T-lymphocyte surface antigen Ly-9-like, detected in the recovered ibexes as compared to the severely infected ones (Supplementary material Table 2).

However, if the local cellular immune response fails to control the infestation in the skin, the infestation

Table 6 Differentially enriched pathways between the recovered and the severely infested Iberian ibexes (*Capra pyrenaica*)

	Expression changes as compared to severely infested				
	SKIN			BLOOD	
		n	Pathways	n	Pathways
Recovered	Up	5	- **Antigen processing and presentation** - Cell cycle - Mismatch repair - Spliceosome - Homologous recombination	0	
	Down	12	- Peroxisome - PPAR signalling pathway - Valine leucine and isoleucine degradation - Galactose metabolism - Fatty acid metabolism - Fructose and mannose metabolism - Glycosaminoglycan degradation - Starch and sucrose metabolism - Tryptophan metabolism - Glycolysis gluconeogenesis - Adipocytokine signalling pathway - Olfactory transduction	21	-Leishmania infection -Toll-like receptor signalling pathway -NOD-like receptor signalling pathway -Fc gamma R mediated phagocytosis -ERBB signalling pathway -Vibrio cholera infection -Chemokine signalling pathway -Epithelial cell signalling in Helicobacter pylori infection -Cytokine-cytokine receptor interaction -Lysosome -Glycerophospholipid metabolism -JAK/STAT signalling pathway -Glutathione metabolism -Ether lipid metabolism -Prostate cancer -Renal cell carcinoma -N glycan biosynthesis -Non-small cell lung cancer -Fc epsilon RI signalling pathway -Glioma -Acute myeloid leukaemia

Pathways determined using the KEGG collection in the GSEA analysis (nominal p-value < 0.05 and FDR < 0.1) in skin and blood. In bold, pathways related with immunity and inflammation

spreads. Such spread seems to overwhelm the systemic immune response, which is not capable to achieve an efficient control of mange despite the increased expression of inflammatory and immune response processes in blood, as previously reported in a porcine model [7, 16]. *Sarcoptes scabei* has adapted genetically to permanent parasitism [79]. For example, to avoid immune cellular recognition, *S. scabiei* mites stimulate the secretion of specific cytokines [80, 81] and down-regulate the gene expression of the immune response in the spleen, including the antigen-presenting cells [13] and inducing local immune suppression in the host to favour mite proliferation and the establishment of the infestation [7, 16]. This agrees with the up-regulation of cytokine production genes (Tables 4 and 5, Supplementary material Tables 2 and 3) and gene sets (Tables 6 and 7) in the blood of the severely infested. This includes, for example, the genes interleukin 18, interleukin 1, 15, and 18 receptors, tumour necrosis factor, tumour necrosis factor ligand superfamily members 9 and 13b, NFAT activating protein with ITAM motif 1, granulocyte–macrophage colony stimulating factor receptor subunits, triggering receptor expressed on myeloid cells 1 (Supplementary material Table 3), and the cytokine-cytokine receptor interaction, NOD-like receptor signalling pathway, toll-like receptor signalling, and complement and coagulation cascades (Table 7), when comparing with the control; and the genes natural killer cells antigen CD94-like and chemokine ligand 5 (Supplementary material Table 2) and the toll-like receptor signalling, NOD-like receptor signalling, chemokine signalling and cytokine-cytokine receptor interaction pathways (Table 6) when comparing with the recovered ibexes.

When comparing both to the control and to the recovered ibexes, additional changes in the gene expression of the severely infested ibexes corresponded with the clinical signs and skin changes associated with severe mange forms (scaling, alopecia, papules, hyperkeratosis, crusts and lichenification) [1]. Such changes included down-regulated expression of more than 40 keratin-related genes, epithelial growth factor 1, fibroblast growth factors 2 and 5, mesoderm specific transcript, trichohyalin, and cornulin, among others (Supplementary material Tables 2 and 3). Depigmentation is also observed in crusted scabies [82, 83], agreeing with the up-regulated processes related with pigmentation found in the control and recovered ibexes as compared to the severely infested ones (Tables 4 and 5). Thus, the severely infested ibexes had lower expression of genes such as premelanosome protein, melanocortin 5 receptor, melanoma-associated antigen D2, and oculocutaneous albinism II among others (Supplementary material Tables 3 and 4), all of them related to skin pigmentation. Accordingly, processes related with response to radiation were also found in the control and in the recovered ibexes (Tables 4 and 5), since pigmentation is influenced by ultraviolet radiation [84]. Depigmentation may affect ibex behaviour towards sunlight in the semiarid mountain environment of Sierra Nevada, which has a high seasonal insolation [85, 86]. The consequent bias in geographical distribution may affect the detectability of Iberian ibex for population

Table 7 Comparison of the pathways upregulated in the skin and blood of the Iberian ibexes (*Capra pyrenaica*) experimentally infested with *Sarcoptes scabiei* as compared to the control

	Upregulation as compared to controls			
	SKIN			**BLOOD**
	n	**Pathways**	**n**	**Pathways**
Recovered	12	- **Antigen processing and presentation** - **Cytokine-cytokine receptor interaction*** - Autoimmune thyroid disease - **NOD-like receptor signalling pathway*** - JAK/STAT signalling pathway - **T-cell receptor signalling pathway** - Prion diseases - **Intestinal immune network for IgA production** - Graft versus host disease - Allograft rejection - Type I diabetes mellitus - Asthma	1	- Olfactory transduction
Severely infested	0		8	-Cytokine-cytokine receptor interaction* -Leishmania infection -NOD-like receptor signalling pathway* -Toll-like receptor signalling pathway -Epithelial cell signalling in Helicobacter pylori infection -Complement and coagulation cascades -JAK/STAT signalling pathway -Lysosome

The differences between the recovered and the severely infested ones as compared to the controls are shown. Pathways determined using the KEGG collection in the GSEA analysis (nominal *p*-value < 0.05 and FDR < 0.1) in skin and blood. In bold, pathways related with immunity and inflammation. The asterisks indicate the pathways upregulated both in the skin of the recovered ibexes and in the blood of the severely infested ones as compared to the controls

monitoring and health surveillance, and must be taken into account for integrated wildlife monitoring [87–90].

This study faces nonetheless at least two major limitations. First, gene expression was only analysed at the disease resolution stage, while studying gene expression at least at three different time points (prior to infestation, at clinical sign onset, and at disease resolution) would have allowed to understand the trend of the local and systemic gene expression responses to the experimental infestation of Iberian ibexes with *S. scabiei* all throughout the disease course. Secondly, the ovine gene microarrays used in this study have not been previously used nor validated in Iberian ibex, which could cause concern regarding the validity of the results obtained. However, genomic tools developed for domestic species can be a powerful tool for analysis of related species [91], and particularly DNA microarrays are reliable to study within-species gene expression for closely related species without discernible loss of information [92, 93]. Iberian ibex is closely related to small domestic ruminants, hybridizing with domestic goats [94]. Therefore, since this study focused on one-time point within species comparison, the results can be considered a significant contribution to the knowledge of the gene expression basis for sarcoptic mange pathogenesis, not only in Iberian ibex but also for other species.

Conclusions

To summarize, this study provides the first insights in the genomic response of Iberian ibex parasitized by *S. scabiei*. The local up-regulation of immune response pathways in the skin was related to the control of the infestation, suggesting a local effective immune response probably related with the antigen processing and presentation function and, therefore, with the activation of T-cells. Conversely, the ibexes with severe sarcoptic mange had an increased systemic inflammatory and immune response, failing to respond effectively to *S. scabiei* infestation. Altogether, compared with the non-infested ibexes, the infested ones had an increased expression of inflammatory and immune processes both in skin and

blood, and a down-regulation of processes related with cell division, independently of the clinical outcome. This first both skin local and blood systemic assessment of the host genomic response to *S. scabiei* infestation will be useful not only for Iberian ibex and other wildlife species, but can also serve as a model for domestic animals and even for humans.

However, further research is required to clarify the immune mechanisms providing protection and resistance against sarcoptic mange. Such studies should include immunohistochemical and molecular analysis to elucidate the cellular and humoral immune response to sarcoptic mange, both locally in skin and systemically in peripheral blood, as well as determining both the genomic and immune responses in earlier stages of the disease, ideally before and at the peak of the infestation, to complete the information of the recovery stage generated in this study.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10999-4.

Supplementary Material 1. Supporting information Figure S1. Trend of the mean percentage of whole-body skin surface affected by lesions compatible with sarcoptic mange in the three experimental groups of Iberian ibexes (*Capra pyrenaica*), namely control, recovered, and severely infested, throughout the experiment.

Supplementary Material 2. Supporting information Figure S2. Number of differentially expressed genes among the different groups of Iberian ibexes (*Capra pyrenaica*) according to sarcoptic mange evolution.

Supplementary Material 3. Supporting information Figure S3a. Number of differentially enriched gene sets and percentage included in pathogenically significant processes between the infested Iberian ibexes (*Capra pyrenaica*) altogether and the controls. Data obtained using the GO biological process collection in the GSEA analysis (nominal *p*-value < 0.05 and FDR < 0.1) both in skin and blood. Supporting information Figure S3b. Number of differentially enriched gene sets and percentage included in pathogenically significant processes between the recovered ibexes and the controls. Data obtained using the GO biological process collection in the GSEA analysis (nominal *p*-value < 0.05 and FDR < 0.1) both in skin and blood. Supporting information Figure S3c. Number of differentially enriched gene sets and percentage included in pathogenically significant processes between the severely infested ibexes and the controls. Data obtained using the GO biological process collection in the GSEA analysis (nominal *p*-value < 0.05 and FDR < 0.1) both in skin and blood

Supplementary Material 4. Supporting information Figure S4. Number of differentially enriched gene sets and percentage included in pathogenically significant processes between the recovered Iberian ibexes (*Capra pyrenaica*) and those severely infested. Data obtained using the GO biological process collection in the GSEA analysis (nominal *p*-value < 0.05 and FDR < 0.1) both in skin and blood

Supplementary Material 5. Supporting information Figure S5. Differentially enriched pathways between the recovered and the severely infested Iberian ibexes (*Capra pyrenaica*) observed using the KEGG collection in the GSEA analysis (nominal *p*-value < 0.05 and FDR < 0.1) in skin and blood.

Supplementary Material 6. Supporting information Figure S6. Comparison of the pathways upregulated in the skin and blood of the Iberian ibexes (*Capra pyrenaica*) that tecovered and the severely infested ones as compared to the controls, using the KEGG collection in the GSEA analysis (nominal *p*-value < 0.05 and FDR < 0.1) in skin and blood.

Supplementary Material 7. Supporting information Table S1. Differentially expressed genes related to pathogenically significant processes between the control and the infested ibexes. The genes were considered differentially expressed for $p<0.05$ and log fold-change -0.58> <0.58 (absolute fold change >1.5), both for skin and blood. The gene up-regulated both in skin and blood is indicated in bold.

Supplementary Material 8. Supporting information Table S2. Differentially expressed genes related to pathogenically significant processes between the recovered and the severely infested ibexes. The genes were considered differentially expressed for p<0.05 and log fold-change -0.58> <0.58 (absolute fold change >1.5), both for skin and blood. The genes either up-regulated or down-regulated both in skin and blood are indicated in bold.

Supplementary Material 9. Supporting information Table S3. Differentially expressed genes related to pathogenically significant processes between the control and the severely infested ibexes. The genes were considered differentially expressed for $p<0.05$ and log fold-change -0.58> <0.58 (absolute fold change >1.5), both for skin and blood. The genes up-regulated both in skin and blood are indicated in bold.

Supplementary Material 10. Supporting information Table S4. Differentially expressed genes related to pathogenically significant processes between the control and the recovered ibexes. The genes were considered differentially expressed for $p<0.05$ and log fold-change -0.58> <0.58 (absolute fold change >1.5), both for skin and blood.

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JEG and JRLO designed the study; ARB, JEG, JE, JMP, FJCM, PF and JRLO performed the experimental infestation, sampling, and curated the samples and data; LN, EP, and MB performed the genomic analyses; ARB, JE, LN, ES and RCS analysed the data; JMP, RCS and JRLO acquired funding; ARB, LN, and JRLO drafted the original version of the manuscript; JEG, EP, RCS and PF revised and supervised the manuscript; all the authors reviewed, significantly contributed to and approved the final version of the manuscript.

Authors' contributions

JEG and JRLO designed the study; ARB, JEG, JE, JMP, FJCM, PF and JRLO performed the experimental infestation, sampling, and curated the samples and data; LN, EP, and MB performed the genomic analyses; ARB, JE, LN, ES and RCS analysed the data; JMP, RCS and JRLO acquired funding; ARB, LN, and JRLO drafted the original version of the manuscript; JEG, EP, RCS and PF revised and supervised the manuscript; all the authors reviewed, significantly contributed to and approved the final version of the manuscript.

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Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

This study complied with all Andalusian, Spanish and European legal requirements and guidelines regarding experimentation and animal welfare. The handling procedures and sampling frequency were designed to reduce stress and minimize the impact on the health of the subjects, as per European (2010/63/UE) and Spanish (R.D 53/2013) standards. The study was approved by the Ethics on Animal Welfare Committee of the University of Jaén and authorized by the Dirección General de Producción Agrícola y Ganadera of the Consejería de Agricultura, Pesca y Medio Ambiente of the Junta de Andalucía (Ref: SA/SIS/MD/ps/ October 25, 2012). The Sierra Nevada Natural Park staff also approved this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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