A piece by Mike Tempest.

AIMÉE VERNALL-FRIDAY, 10 JANUARY 2020-12 MINUTES

QUESTIONS FOR AUTHORS OF PAPER ENTITLED "POPULATION STRUCTURE AND GENETIC HISTORY OF TIBETAN TERRIER" from Dr Mike Tempest (Mikudi TTs)

(1) What thought processes made you conduct this study? The TT would be a relatively minority insignificant breed in Croatia and Slovenia, so I am curious as to what possible interest geneticists could have had in the TT breed, enough to make it the subject of a major study?

(2) ABSTRACT In the Abstract you state that you analysed the structure of the original native population. Could you please explain how you did this when you could not possibly have obtained DNA from the original native population that provided the dogs that were brought to the west. Do you mean that you analysed the current native population which is almost 100 years on from the original dogs?

(3) BACKGROUND You state various matters in the Background section of your paper that are not correct.

(a) You state that Bunti and Rajah were acquired by Dr Greig in 1922. This is not correct. 1922 was the year in which Dr Greig first made an acquaintance with a Tibetan Terrier when a Tibetan man arrived at the hospital where Dr Greig worked in Cawnpore together with his sick wife and his animals, one of which was a bitch called Lily. Dr Greig cared for Lily in her home until the wife was well enough to go home. Rajah was never acquired by Dr Greig. Lily was the dam of Bunti later given to Dr Greig for saving the life of the Tibetan man's wife.

(b) You state that Bunti and Rajah were brought into England in 1930. The UK's historical records show that Rajah was not brought to England, he was brought to Dr Greig by some of her Tibetan friends in Tibet with the sole purpose of mating Bunti in Tibet. They produced two litters in Tibet born on Christmas Day 1924 and in July 1925. Bunti was not brought to England in 1930, she was brought to England in 1926 when Dr Greig returned to England on home leave. At the same time Dr Greig brought to England a bitch, Chota Tukra, from Bunti's first letter by Rajah, and a dog, Ja-Haz, from her second litter. These three Tibetan Terriers were accepted for registration by the British Kennel Club as Lhasa

Terriers, since that was their classification in India. The registrations appear in the records section of the UK Kennel Club's Kennel Gazette of December 1926 with Dr Greig's mother named as the owner and Miss (Dr) A. R. H. Greig as the breeder. The records were later corrected as Dr Greig did not breed Bunti. From where did you obtain the incorrect information about Bunti and Rajah?

(c) You state that the Kennel Club of England recognised the Tibetan Terrier as an own breed in 1937. This is incorrect – it recognised the Thibetan Terrier as a separate breed in 1930, and it recognised the Tibetan Breeds Association in 1934. Again, from where did you obtain the incorrect information about this?

(d) You state the English Kennel Club's recognition of the TT was based on animals belonging to the Lamleh lineage. It was not – all the early TTs were registered under Mrs Greig's kennel name of Ladkok.

(e) There were no TTs registered as 'Lamleh' until 1932, when Dr Greig's new kennel name of 'Lamleh' was added to two dogs which Dr Greig had acquired (Gyan Tse and Yukshee) but which were bred by others, and applied to one dog she had bred (Shiga of Lamleh), all of which were owned by Mrs Greig. Dr Greig did not own any TTs in her name until 1934.
(f) The dog 'Dusky', registered as Trojan Kynos in April 1953, was found by John Downey on the dockside in Morecambe, not in Liverpool as you state.

(g) You state that "both lineages [i.e. Lamleh and Luneville] go back to a very limited number of founders from the native Tibetan Terrier (TTNA) population and represent the link between the western population of Tibetan Terriers and the original population in Tibet". This is not true of the 'Luneville lineage' as there is no evidence that the dog Trojan Kynos, found on a dockside in England, had any link with the native Tibetan Terrier population, nor is there any evidence that dogs brought to England from Nepal and India by people other than Dr Greig had any link with the native Tibetan Terrier population. They were all 'Pedigree Unknown' which means there is no evidence of their ancestry/genetic background.

(h) You could have had this accurate record if you had been directed to the authoritative reference book on TTs authored by Angela Mulliner, Volumes 1 and 2, published in 1977, re-printed 1991, printed by Holywell Press, Oxford, and I am curious as to why you have not used this reference.

(i) You could also have been provided with accurate information by the 'mother country' i.e. the country where the breed was established and developed, recognised by FCI as the 'Country of Patronage'. Both the English Kennel Club and the founder breed club, the Tibetan Terrier Association, would willingly have provided this information, but neither organisation was aware of your study. The Tibetan Terrier breed holds World Congresses every 2-3 years, but no mention has ever been made at these congresses, the latest in 2017, of your study, which gives the impression that it has been kept secret from the world TT fraternity. Why did you not consider it necessary to involve these senior organisations?
(j) You state that "The western population is registered at the FCI". Firstly, the FCI is not a dog registry – its member countries have their own registries. Secondly the entire western population is not registered with FCI, nor with the totality of all FCI member clubs. The largest registered western population is with the English KC, but you do not recognise it. Another large registry is the American Kennel Club which you do not mention. Why did you not involve these two large registries?

(k) You state that it is only recently that a reproductive contact has been made between the western population and the native Tibetan population. This is not true. Such reproductive contact happened 50 years ago, halfway through the history of the breed in the west. In 1967 (registered September 1967), a bitch called 'Tilly', which had been born in Tibet, was brought in from Assam by Mrs Aileen Smith, and was later registered as 'Deki La Chenga' (meaning the Charming Unknown One). Later in 1967 a bitch called 'Sindie' registered 'Anjuman Sindie' (in November 1969) was brought in from Tibet via Nepal by Mrs Angela Mulliner. These two bitches produced offspring that founded the 'Anjuman' and 'Kangri' breeding lines and both made a major genetic contribution to the breed through breeding combinations with both 'Lamleh' and 'Luneville' dogs, which were then intermixed. (1) You state that it is only very recently that reproductive contact between both populations [native and western] has occurred through a limited number of imported dogs from Tibet to western countries. Could you please be specific and state who these dogs are and to which western countries they were imported, and who their owners are? Which of these recent imports from Tibet were used to produce your 8 F1 crosses of native to Lamleh? (m) You state in the paper that the original gene pool is still present in Tibet and represents the original gene pool from which a few individuals contributed as founders to the western

population. Do you not accept that the original gene pool, from which came the first imports to the west, will have changed genetically in the 100 years since those imports? Furthermore, the original imports came from two different sources – the herding type of the nomadic herdsmen of the steppe (e.g. Bunti) and the monastery type of the mountains (e.g Thoombay of Ladkok). These two types were slightly different, because of climate and terrain, and the more settled lifestyle in the monasteries compared to the wandering lifestyle of the nomads. Did you take this into account in your paper?

both long-legged and short-legged dogs in the same litter. The Shih Tzu was developed in China from Lhasa Apsos traded on the silk route and selected in China for heads that were like the 'Lion' statues, thus becoming known as the 'Little Lion Dog'. The Tibetan Spaniel is likely to have been developed from a recessive short-haired Lhasa Apso, known as a 'perhapso'! I know of no evidence which shows it was the TT that contributed to the formation of the gene pool of the other breeds.

(o) The word 'terrier' was used only to name the breed because it was not known what other word could be used ('apso' simply means long coated). There has never been any suggestion that TTs were sometimes bred from terriers. No responsible breeder would do this intentionally or deliberately, and I think your claim that there might have been a few sporadic crosses between Tibetan Terriers and Terriers during the last century cannot be substantiated by evidence – indeed you phrase it as a 'might be due to' which is not evidence.

(p) You state that your goal of your study was to provide an insight into the genomic structure and genetic history of TTs. In the Abstract your priority conclusion seems to be to reject the original hypothesis that TTs belong to the group of Terrier breeds and support the hypothesis that TTs belong to the group of ancient dog breeds of Asian origin. Do you consider that this is a significant original finding, or might we have known that already?

(4) METHODS I have no issue with the genotypic techniques which you used, but I have some questions about Sampling on which the whole study depends.

(a) How did you identify 'Lamleh' and 'Luneville' lineages?

(b) Of the 24 'western Tibetan Terriers', 20 were 'Lamlehs' but only 4 were 'Lunevilles'. Do you

not consider that this biased your study towards 'Lamleh'?

(c) There is no indication in the 'Methods' section of the paper of the countries of residence of

the sampled TTs of the 'western TT population'. Could you please supply this information and the details and pedigrees of the 24 'western TTs', and say how and by whom they were selected?

(d) In the 'Methods' section it says that 22 native TT samples were collected at 22 locations in Tibet. How and by whom were these dogs selected for sampling when there is no ancestry or pedigree information on them? They could well have been crossbreds that had specific morphological criteria that made them look like TTs! It is difficult to accept that these were purebred when there is no evidence of such, nor is it possible to acquire such evidence.

(e) If the samplers could go as far as to Tibet, why could they not come to the UK or America to sample dogs?

(f) There were also a number of crosses between 'native' dogs and 'Lamlehs' – 8 F1s, and 10 backcrosses (BC) (6 F1 x Lamleh called BC2, and 4 BC2 x Lamleh called BC3); but no crosses between 'natives' and 'Lunevilles'. This a strange omission, and it is valid to ask why?

(g) How were the native crosses sourced? Were they crosses with the native population in Tibet that were sampled; or were the 8 F1s x all bred from one recent native import?

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(5) RESULTS
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(a) You say you detected significant gene flow from the native population to both Lamleh and

Luneville lineages. Would you consider that this is 'not surprising'? Western breeders know that Lamleh and Luneville were 'mixed together' in the very early days of breed development. For example, 'Luneville Prince Khan', the primary stud dog of the Luneville

kennel, was sired by a Lamleh dog out of a bitch called 'Luneville Lady Penelope' whose maternal grandfather was another Lamleh dog!

(b) Would you consider that your result that Western TTs show more uniformity than native TTs is again not surprising? Western TTs have been selectively bred, native TTs are the result of random matings some of which are very likely to have been 'crossbred' random matings in the street.

(c) You state that "the existence of a broader genetic pool in the native population of TTs is highly relevant for possible prevention measures against ... the risk for genetic diseases". Presumably in order to make this statement you have tested the native population and found them to be free of genetic diseases (unspecified). Could you please supply the evidence which supports your statement?

(6) DISCUSSION

(a) You state that the native ancestral population is available in Tibet. I would dispute this on

the grounds that the current native population is 100 years further on than the original natïve ancestral population. You have not DNA sampled the original native ancestral population, only the current native population which may have provided a recent import into the west, and the current population cannot possibly represent the original gene pool of 100 years ago. I would be grateful for your comments on this.

(b) Whilst I agree that the breed experienced considerable inbreeding on the original imports into the UK by Dr Greig, I would have to question your assertion that this led to a rapid increase in hereditary health problems. The health problems that we are now aware of were not known at the time of inbreeding on the original imports. The health problems cannot be blamed entirely on the western population which was established on the original imports. Furthermore, inbreeding will not cause hereditary health problems, it will only bring them to the surface if they are genetically present in the first place.

(c) I dispute your assertion that when the western population of TTs was established it was placed within the Terrier group. I am confused by your conflicting statements "that your results confirm that the dog known today as TT is not a terrier at all" and "a contribution of Terriers to the western population can be assumed". Do you not consider that scientific

research should provide 'proof based on evidence' and should not promulgate 'assumptions'?

(d) Why do you assume that the intermediate position of the F1 individuals and the position of the two backcross generations clearly show the effect of genetic drift in the Lamleh lineage and not to genetic drift in the native population? Other genetic material from imports from Nepal and India were introduced into Lamleh very early in its breeding programme.

(e) The very low proportion of the genome in ROH for the native population could have been a consequence of random matings with 'street dogs' i.e. the native population was a mixture and was not necessarily a genetically diverse purebred TT population. You do not seem to have recognised this feature of life in Tibet 100 years ago, when breed purity was unheard of.

(7) SUPPLEMENTARY INFORMATION

Additional File 2: Table S1. Could you explain why the number of dogs that were included in the analyses used in this study are different from the number given in the Methods Sampling section. In Additional File 2 Table S1 it gives the numbers as: Tibetan Terriers 7 (it does not state which population were these from), TTF1 5, TTLA 5, TTLU 1, and TTNA 7. Could you please explain the discrepancy and what population the7 dogs just described as Tibetan Terriers were from?

Competing interests. You acknowledge that as authors you have no competing interests. Could the same be said of the two people you acknowledge for providing selfless assistance and expert guidance in sample collection, who are both well-known in the TT fraternity as supporters of 'Lamleh'?