

RESEARCH PAPER

Don't it make your brown eyes blue? A comparison of iris colour across latitude in Australian twins

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Modern migration of Europeans to New World countries and to a lesser extent, Africans and Asians to European countries, has resulted in people living in environments that differ from their evolution of the last 100,000 years.¹ For example, this has led to a higher prevalence of malignant melanoma in Europeans who have moved to Australia, especially in areas closer to the equator (for example, Queensland). It is often presumed that Europeans migrating to countries with latitudinal spans that encompass both tropical and temperate regions (for example, United States at 52°, Argentina at 33°, Australia at 32°) randomly moved to different parts of the country; however, the social process of chain or serial migration frequently determined the final settlement location; people from one region in the home country often moved to the same area in the destination country (albeit not necessarily at the same latitude).

Background: The aim was to determine whether latitudinal (Queensland versus Tasmania) variation in reported disease frequency in Australia may be biased by differences in population.

Methods: A retrospective analysis was conducted from data of two large Australian twin studies ($n = 1,835$) having undertaken ophthalmic examination, namely, Twins Eye Study in Tasmania (TEST) and the Brisbane Adolescent Twins Study (BATS). Ordinal logistic regression was used to compute odds ratios and predicted probabilities for each category of eye colour by state.

Results: Tasmanian residence was associated with lower odds of darker iris colour (odds ratio 0.77, 95% CI [0.63–0.95]) signifying that participants living in Tasmania (TAS) are less likely to have darker-coloured irides than those residing in Queensland (QLD). For individuals living in Tasmania the predicted probability (TAS versus QLD) of having light blue eyes was greater (16.7 versus 13.3 per cent), approximately the same for green eyes and less for brown/dark brown-coloured eyes (6.2 versus 7.9 per cent).

Conclusions: We found a general trend of individuals living in the southern states (TAS/VIC) of Australia having lighter-coloured irides compared to those living in the north (QLD). This finding has potential implications for all epidemiological research conducted to explore differences in UV-associated disease frequency in Australia, as population heterogeneity may confound the estimates obtained.

Many epidemiological studies have attempted to elicit relationships between disease prevalence and UV exposure along latitudinal gradients. These have comprised not only investigations of disease arising from the carcinogenic effects of excessive UV but also those due to vitamin D deficiency associated with inadequate UV exposure. For example, the pathogenic effect of UV in the development of skin cancers and cutaneous malignant melanoma is well recognised.^{2,3} Conversely, a 'protective' role of UV has been hypothesised for various cancers (prostate, colorectal, breast), mental illness and autoimmune disorders (multiple sclerosis, type II diabetes), whereby exposure to UV at lower latitudes ensures sufficient biosynthesis of vitamin D in the skin for human health.^{4,5} Paradoxically, compared to the cutaneous form, the incidence of uveal melanoma has been observed to follow an inverse latitudinal

gradient, that is, increasing incidence commensurate with latitude.⁶

Such research has usually been predicated on the assumption of population homogeneity across the latitudinal gradient being considered; however, if populations have already 'self selected' their preferred latitude, this work may actually underestimate the impact of UV exposure as the population effect confounds estimates of disease frequency. No data have been published comparing skin, hair or iris colour of people living at different latitudes in Australia. Studies of different UV exposure in the southern states of Australia (for example, Tasmania) compared to the north (for example, Queensland) have presumed latitudinal homogeneity.⁷ In this study we used a large cohort of twin participants to determine whether there were any differences in the frequencies of different eye colours between individuals living in Tasmania

compared with those living in Queensland. Using eye colour as a proxy for genetic heterogeneity, this would allow us to determine whether latitudinal variation in reported disease frequency in Australia may be biased by differences in population.

METHODS

Participants

Twins were recruited and examined as part of the Twins Eye Study in Tasmania (TEST)⁸ and the Brisbane Adolescent Twin Study (BATS).⁹ For both cohorts the vast majority of participants were of reported Northern European ancestry and consisted of predominantly younger individuals of each sex. Recruitment was conducted without providing subjects with knowledge of specific hypotheses or eye studies being conducted, reducing ascertainment bias. Subjects provided informed consent for their participation in this study and their data were anonymised for analysis. The relevant ethics committees of the Royal Victorian Eye and Ear Hospital and University of Tasmania approved the study. The study adhered to the tenets of the Declaration of Helsinki.

Categorisation and grading of iris colour

To obtain eye colour data, digital photographs of participants' mid-face including both irides were taken with a Nikon Coolpix 995 camera (Nikon, Tokyo, Japan) at a standardised distance of 30 cm using the internal camera flash (in addition to standard room illumination). Photographs of both eyes were used to categorise an individual's eye colour. The categorisation of eye colour for this study was based on the original three-scale characterisation of eye colour used in the BATS¹⁰ and then refined to include intervening categories, giving nine iris colour categories consisting of (light to dark):¹¹

1. Light blue
2. Darker blue
3. Blue with brown pupil ring
4. Green
5. Green with brown iris ring
6. Peripheral green central brown
7. Brown with some peripheral green
8. Brown and
9. Dark brown.

The 'dark brown' category was created to accommodate the heavily pigmented

Melanesian/East Asian and African irides; however, only six participants in the sample were classified as this and therefore, for statistical purposes we collapsed categories 8 and 9 together.

To grade iris colour, one observer sorted digital images of participant mid-face photographs containing both eyes into one of the nine colours. Photographs in each category were then sorted to determine whether the photograph remained in that category or was moved up or down one category. This was sorted by a first grader (DAM), second grader (CHW) and again by the first grader. Final consensus was reached by open discussion between the graders. The vast majority (more than 98 per cent) of photographs were taken prior to instillation of cycloplegic drops, allowing maximal visualisation of the iris. In a small percentage of cases, photographs were taken with the pupil in a dilated state. The effect of pupil dilation on eye colour does not appear to be dramatic with Mackey and colleagues¹¹ showing no change in eye colour (11/15 or 73 per cent) for a small sample of subjects re-assessed following dilation.

Statistical analysis

Ordinal logistic regression modelling was used to analyse the relationship between iris colour and geographical location. An extension of the binary logistic regression model, the proportional odds (or cumulative logit) model allows for more than two (ordered) response categories.¹² Rather than considering the categorisation nominal, we chose to view eye colour within an ordered classification scheme, based on the underlying notion that a continuum of eye colour exists determined largely by quantitative differences in the density and size of iris melanosomes.¹⁰ In this way, the proportional odds model allows eye colour to be analysed as a series of binary comparisons: category 1 versus categories 2–8/9, categories 1–2 versus categories 3–8/9, . . . categories 1–7 versus categories 8/9 et cetera, such that seven cumulative logits are modelled for the eight levels of iris colour represented. Consequently, the odds ratio computed for a predictor may be interpreted as a summary of the odds ratios determined from independent binary logistic regressions using all possible threshold (cutoff) values of the ordinal outcome. In addition to reporting odds ratios, predicted probabilities were calculated for each category of iris colour. Statistical analyses were carried out with

Stata version 12.0 (2011, StataCorp, College Station, Texas, USA) using the *ologit* command. To account for familial clustering of twin data, analyses were conducted using the *cluster* option in Stata to generate robust standard errors and confidence intervals.¹³

An important assumption underlying the proportional odds model is that the relationship between each pair of outcome categories is statistically the same. In effect, this assumes that the regression coefficients that describe the association between, for example, category 1 versus categories 2–8/9 of iris colour is equal to that for categories 1–7 versus 8/9. Validation of the assumption enables one set of coefficients (that is, one model) to be specified and was tested in Stata using the *omodel* command and a likelihood ratio (LR) test (the null hypothesis being that there is no difference in the coefficients between models).

RESULTS

The mean and standard deviation of age across all participants was 28.9 ± 13.4 years (range 9 to 99 years), with females accounting for 56.1 per cent of the cohort. There were 1,067 families represented in our dataset, with 969 who had at least one member with iris colour data recorded. All members of each family resided within the same state. Iris photographs were available for 1,835 participants and a summary of the frequencies of different iris colour categories is presented in Figure 1A. Over one-half of the participants (52.9 per cent) were from Queensland, 742 (40.6 per cent) from Tasmania and a further 121 (6.6 per cent) resided in Victoria. As the number of participants from Victoria was small and the latitudinal difference between southern states nominal compared to that of Queensland, Victorian participants were grouped with those from Tasmania for the purpose of subsequent analyses.

Two ordinal logistic regression models were evaluated. In the first instance, the effect of state alone on iris colour was determined and then the model was recomputed with age and sex included as fixed effects. Model fitness was assessed with the Wald test; the probability ($p > \chi^2$) of observing a Chi-square statistic as extreme as that observed under the null hypothesis, in which the coefficients for age and sex are equal to zero, was calculated. Age and sex were found not to contribute significantly to the prediction of

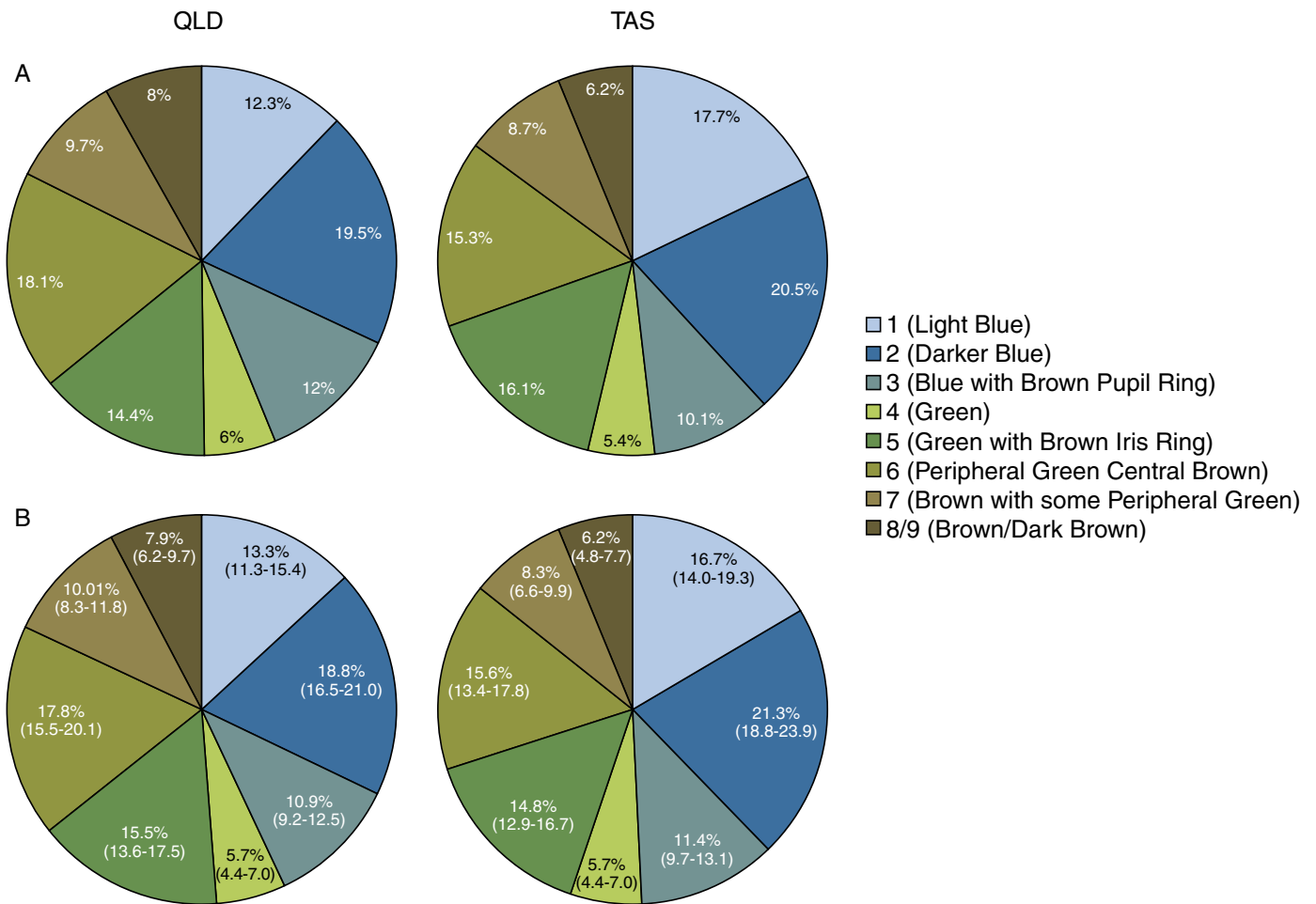


Figure 1. Observed (percentage frequency) (A) and predicted probabilities (percentage [95% CI]) (B) for iris colour by state.

iris colour (Wald $\chi^2 = 2.6$, $df = 2$, $p = 0.27$) and therefore, were removed from subsequent model fitting. The proportional odds assumption was observed to be valid (approx. LR $\chi^2 = 7.0$, $df = 6$, $p = 0.32$) enabling the parameter coefficient for state to remain equal across all ordered pairings of iris colour categories. A test of the full model against a constant only model was statistically significant, indicating that state is a predictor of variation in iris colour ($\chi^2 = 5.84$, $df = 1$, $p = 0.016$). The results of the regression analysis showed that Tasmania was associated with a lower odds of darker iris colour (OR 0.77, 95% CI [0.63–0.95]; QLD=0, TAS=1) signifying that participants living in Tasmania are less likely to have darker coloured irides than those residing in Queensland.

The predicted probabilities for each category of iris colour contrasted by state are shown in Figure 1B. For individuals living in

Tasmania, the predicted probability (TAS versus QLD) of having light blue eyes is greater (16.7 versus 13.3 per cent), approximately the same for green eyes and less for brown/dark brown-coloured eyes (6.2 versus 7.9 per cent). Thus, the data reflect a general trend of lighter-coloured irides in individuals living in the southern states (TAS and VIC) of Australia compared to those living in the north (QLD).

DISCUSSION

The results of our study have shown for the first time a statistically significant difference in the distributions of eye colour across two latitudinally diverse locations in Australia. Using a twin population, we ascertained that individuals living in the southern states of Australia tend to have lighter coloured eyes than their northern-based compatriots. This finding has potential implications for all epi-

demiological research conducted to explore differences in UV-associated disease frequency in Australia, as population heterogeneity may confound the estimates obtained.

Aside from eye colour, variation in skin pigmentation remains the most obvious human phenotype to distinguish individuals based on geographic and ethnic differences. While epidemiological data are available for other regions, information relating to variation of these traits within Australia is notably lacking. Evolutionary pressures are thought to have driven latitudinal differences in skin pigmentation; people living near the equator tend to have darker skin compared to those nearer the poles.^{14,15} At a biological level, this may be explained in terms of vitamin D synthesis, whereby the protection provided by dark skin from UV irradiation becomes a liability at higher latitudes with lower levels of exposure. In contrast to that for skin colour, there are no clear

environmental selective pressures for the variation observed in eye colour, although one hypothesis has implicated sexual selection given that it favours colour traits and polymorphisms.¹⁶ Certainly, a significant genetic basis for eye colour exists, with the *OCA2* gene on chromosome 15 estimated to explain 74 per cent of the variance in the trait and in European populations being primarily associated with blue eyes.¹⁰ In addition, research has shown that gene-gene interactions contribute to the variability of pigmentation in humans; for example, the *HERC2* gene located upstream of *OCA2* being associated not only with eye colour but also hair colour and skin type.¹⁷

Data from our study suggest some non-randomness to the Australian population in eye colour with more blue eyes in Tasmania and Victoria compared with Queensland. Compared to its northern hemisphere counterparts, Australia remains a relatively young, sparsely populated country, with the first European settlers arriving in the late 1700s. Since then, many ethnically diverse groups have migrated to different regions within the country and modern research is conducted assuming random migration and population homogeneity. If in fact ethnic groups have undergone some degree of geographical segregation within the country, comparative assessments of UV-associated disease risk between locations may be biased. For example, a 2008 report published by the Australian Institute of Health and Welfare providing comprehensive national data on cancer incidence and mortality did not account for potential regional differences in population makeup.¹⁸ Age-standardised incidence rates for cutaneous malignant melanoma were calculated as 45.5 per 100,000 in Tasmania compared to 65.3 per 100,000 in Queensland. If the pigmentation phenotype (including eye colour) is darker in Queensland compared to Tasmania and thus partially protective for sun damage, it is possible that the harmful effects of UV are erroneously under-reported. In addition to melanoma, incidence estimates for other UV-associated cancers and auto-immune disorders may be similarly affected.

Why are there more blue-eyed individuals living in Tasmania compared to Queensland? Early colonists with fair skin (prior to sunscreen and air conditioning) may have chosen to leave Queensland for Tasmania. Even taking into account the effects of chain migration, this finding appears coincidental. Perhaps migrant groups sought out

similar environmental conditions to that which they were accustomed in the home country (for example, more northern Europeans moving to the southern climes of Australia). Certainly, the country is too recently founded for selective pressures to take effect. In older populations, Sturm and Larssen¹⁶ posit an interesting theory for the latitudinal gradient observed in eye colour. From an evolutionary perspective, they suggest ‘... perhaps those with blue eyes may have been able to withstand the dark, depressing days of the Neolithic European winters better than those with brown eye colour?’¹⁶ Recent work by Goel, Terman and Terman¹⁹ seems to support this premise with their finding that among depressed individuals, those with darker eyes were more depressed and fatigued in the winter months than those with blue eyes.

There are several limitations to our study. It is possible that there may have been selection bias in the studies as the initial twin research in Queensland related to skin cancer risk (although that may have motivated more fairer-pigmented Queenslanders to participate). Participation rates from ethnic minorities tend to be lower and thus, these data do not reflect the true population frequencies of eye colour, but may be more aligned with research participation rates. From a methodological viewpoint, the categorisation of eye colour discards a substantial amount of colour information. Recently, Liu and colleagues²⁰ described a method of quantifying eye colour variation on a continuous scale using hue and saturation values derived from high-resolution digital photographs. Further work in this area may consider using such an approach to improve statistical power.

In summary, we have shown that a small but significant difference in eye colour exists between the northern and southern states of Australia, with more blue-eyed individuals living in Tasmania. This is an important observation for researchers comparing latitudinal variation of UV-associated disease frequency in Australia and should be considered in future work.

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